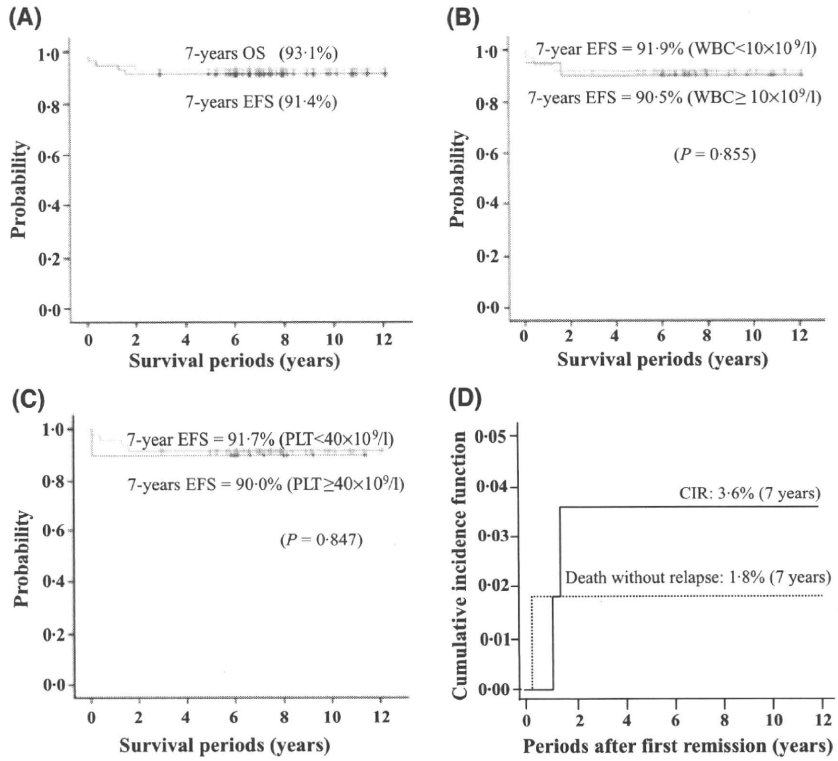

Fig 2. Analysis of the rates of OS, EFS and cumulative incidence functions of CIR and death without relapse in patients treated with the AML99-M3 protocol. (A) OS and EFS rates of total patients; (B) EFS rates of patients with WBC count > 10 × 10^9/l or <10 × 10^9/l at diagnosis; (C) EFS rates of patients 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). [Correction added on 1 October 2010, after first online publication: The data in Figure 2B was amended.]

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, ×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.

Acknowledgements

The authors are thankful to the participating paediatric oncologists in this study for providing the clinical data. We thank Drs Hiroyuki Takahashi and Koichiro Ikuta for supporting this study. This work was supported by a Grant for Clinical Cancer Research and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan.

References

Adès, L., Chevret, S., Raffoux, E., de Botton, S., Guerci, A., Pigneux, A., Stoppa, A.M., Lamy, T., Rigal-Huguet, F., Vekhoff, A., Meyer-Monard, S., Maloisel, F., Deconinck, E., Ferrant, A., Thomas, X., Fegueux, N., Chomienne, C., Dombret, H., Degos, L. & Fenaux, P. (2006) European Acute Promyelocytic Leukemia Group. Is cytarabine useful in the treatment of acute promyelocytic leukemia?

Results of a randomized trial from the European Acute Promyelocytic Leukemia Group. *Journal of Clinical Oncology*, **24,** 5703–5710.

Adès, L., Sanz, M.A., Chevret, S., Montesinos, P., Chevallier, P., Raffoux, E., Vellenga, E., Guerci, A., Pigneux, A., Huguet, F., Rayon, C., Stoppa, A.M., de la Serna, J., Cahn, J.Y., Meyer-Monard, S., Pabst, T., Thomas, X., de Botton, S., Parody, R., Bergua, J., Lamy, T., Vekhoff, A., Negri, S., Ifrah, N., Dombret, H., Ferrant, A., Bron, D., Degos, L. & Fenaux, P. (2008) Treatment of newly diagnosed acute promyelocytic leukemia (APL): a comparison of French-Belgian-Swiss and PETHEMA results. *Blood*, **111,** 1078–1084.

Adès, L., Guerci, A., Raffoux, E., Sanz, M., Chevallier, P., Lapusan, S., Recher, C., Thomas, X., Rayon, C., Castaigne, S., Tournilhac, O., de Botton, S., Ifrah, N., Cahn, J.Y., Solary, E., Gardin, C., Fegeux, N., Bordessoule, D., Ferrant, A., Meyer-Monard, S., Vey, N., Dombret Degos, H.L., Chevret, S. & Fenaux, P. (2010) European APL Group. Very long-term outcome of acute promyelocytic leukemia after treatment with all-trans retinoic acid and chemotherapy: the European APL Group experience. *Blood*, **115,** 1690–1696.

Bagnes, C., Panchuk, P.N. & Recondo, G. (2010) Antineoplastic chemotherapy induced QTc prolongation. *Current Drug Safety*, **5,** 93–96.

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R. & Sultan, C. (1982) Proposals for the classification of the myelodysplastic syndromes. *British Journal of Haematology*, **51,** 189–199.

de Botton, S., Coiteux, V., Chevret, S., Rayon, C., Vilmer, E., Sanz, M., de La Serna, J., Philippe, N., Baruchel, A., Leverger, G., Robert, A., San Miguel, J., Conde, E., Sotto, J.J., Bordessoule, D., Fegueux, N., Fey, M., Parry, A., Chomienne, C., Degos, L. & Fenaux, P. (2004) Outcome of childhood acute promyelocytic leukemia wit all-trans-retinoic acid and chemotherapy. *Journal of Clinical Oncology*, **22,** 1404–1412.

de Botton, S., Sanz, M.A., Chevret, S., Dombret, H., Martin, G., Thomas, X., Mediavilla, J.D., Recher, C., Ades, L., Quesnel, B., Brault, P., Fey, M., Wandt, H., Machover, D., Guerci, A., Maloisel, F., Stoppa, A.M., Rayon, C., Ribera, J.M., Chomienne, C., Degos, L., Fenaux, P.; European APL Group & PETHEMA Group. (2006) Extramedullary relapse in acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. *Leukemia*, **20,** 35–41.

Breccia, M., Carmosino, I., Diverio, D., De Santis, S., De Propris, M.S., Romano, A., Petti, M.C., Mandelli, F. & Lo-Coco, F. (2003) Early detection of meningeal localization in acute promyelocytic leukaemia patients with high presenting leucocyte count. *British Journal of Haematology*, **120,** 266–270.

Chow, J. & Feusner, J. (2009) Isolated central nervous system recurrence of acute promyelocytic leukemia in children. *Pediatric Blood & Cancer*, **52,** 11–13.

Fenaux, P., Chastang, C., Chevret, S., Sanz, M., Dombret, H., Archimbaud, E., Fey, M., Rayon, C., Huguet, F., Sotto, J.J., Gardin, C., Makhoul, P.C., Travade, P., Solary, E., Fegueux, N., Bordessoule, D., Miguel, J.S., Link, H., Desablens, B., Stamatoullas, A., Deconinck, E., Maloisel, F., Castaigne, S., Preudhomme, C. & Degos, L. (1999) A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. *The European APL Group Blood*, **94,** 1192–1200.

Ferrara, F. (2010) Acute promyelocytic leukemia: what are the treatment options? *Expert Opinion on Pharmacotherapy*, **11,** 587–596.

Grignani, F., Fagioli, M., Alcalay, M., Longo, L., Pandolfi, P.P., Donti, E., Biondi, A., Lo Coco, F., Grignani, F. & Pelicci, P.G. (1994) Acute promyelocytic leukemia: from genetics to treatment. *Blood*, **83,** 10–25.

Guglielmi, C., Martelli, M.P., Diverio, D., Fenu, S., Vegna, M.L., Cantù-Rajnoldi, A., Biondi, A., Cocito, M.G., Del Vecchio, L., Tabilio, A., Avvisati, G., Basso, G. & Lo Coco, F. (1998) Immunophenotype of adult and childhood acute promyelocytic leukaemia: correlation with morphology, type of PML gene breakpoint and clinical outcome. A cooperative Italian study on 196 cases. *British Journal of Haematology*, **102,** 1035–1041.

Ko, B.S., Tang, J.L., Chen, Y.C., Yao, M., Wang, C.H., Shen, M.C. & Tien, H.F. (1999) Extramedullary relapse after all-trans retinoic acid treatment in acute promyelocytic leukemia – the occurrence of retinoic acid syndrome is a risk factor. *Leukemia*, **13,** 1406–1408.

Le Deley, M.C., Leblanc, T., Shamsaldin, A., Raquin, M.A., Lacour, B., Sommelet, D., Chompret, A., Cayuela, J.M., Bayle, C., Bernheim, A., de Vathaire, F., Vassal, G., Hill, C. & Société Française d'Oncologie Pédiatrique. (2003) Risk of secondary leukemia after a solid tumor in childhood according to the dose of epipodophyllotoxins and anthracyclines: a case-control study by the Société Française d'Oncologie Pédiatrique. *Journal of Clinical Oncology*, **21,** 1074–1081.

Lenk, H., Tanneberger, S., Wiener, N., Giesske, H., Gärtner, S., Geyer, J. & Rotte, K.H. (1990) Phase II study of pirarubicin (THP-adriamycin) in metastatic breast cancer patients. *Oncology*, **47,** 97–100.

Liso, V., Specchia, G., Pogliani, E.M., Palumbo, G., Mininni, D., Rossi, V., Teruzzi, E., Mestice, A., Coppi, M.R. & Biondi, A. (1998) Extramedullary involvement in patients with acute promyelocytic leukemia: a report of seven cases. *Cancer*, **83,** 1522–1528.

Mann, G., Reinhardt, D., Ritter, J., Hermann, J., Schmitt, K., Gadner, H. & Creutzig, U. (2001) Treatment with all-trans retinoic acid in acute promyelocytic leukemia reduces early deaths in children. *Annals of Hematology*, **80,** 417–422.

Nysom, K., Holm, K., Lipsitz, S.R., Mone, S.M., Colan, S.D., Orav, E.J., Sallan, S.E., Olsen, J.H., Hertz, H., Jacobsen, J.R. & Lipshultz, S.E. (1998) Relationship between cumulative anthracycline dose and late cardiotoxicity in childhood acute lymphoblastic leukemia. *Journal of Clinical Oncology*, **16,** 545–550.

Ortega, J.J., Madero, L., Martín, G., Verdeguer, A., García, P., Parody, R., Fuster, J., Molines, A., Novo, A., Debén, G., Rodríguez, A., Conde, E., de la Serna, J., Allegue, M.J., Capote, F.J., González, J.D., Bolufer, P., González, M., Sanz, M.A. & PETHEMA Group. (2005) Treatment with all-trans retinoic acid and anthracycline monochemotherapy for children with acute promyelocytic leukemia: a multicenter study by the PETHEMA Group. *Journal of Clinical Oncology*, **23,** 7632–7640.

Sakata-Yanagimoto, M., Kanda, Y., Nakagawa, M., Asano-Mori, Y., Kandabashi, K., Izutsu, K., Imai, Y., Hangaishi, A., Kurokawa, M., Tsujino, S., Ogawa, S., Chiba, S., Motokura, T. & Hirai, H. (2004) Predictors for severe cardiac complications after hematopoietic stem cell transplantation. *Bone Marrow Transplantation*, **33,** 1043–1047.

Sanz, M.A., Lo Coco, F., Martín, G., Avvisati, G., Rayón, C., Barbui, T., Díaz-Mediavilla, J., Fioritoni, G., González, J.D., Liso, V., Esteve, J., Ferrara, F., Bolufer, P., Bernasconi, C., Gonzalez, M., Rodeghiero, F., Colomer, D., Petti, M.C., Ribera, J.M. & Mandelli, F. (2000) Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. *Blood*, **96,** 1247–1253.

Sanz, M.A., Martín, G., González, M., León, A., Rayón, C., Rivas, C., Colomer, D., Amutio, E., Capote, F.J., Milone, G.A., De La Serna, J.,

Román, J., Barragán, E., Bergua, J., Escoda, L., Parody, R., Negri, S., Calasanz, M.J., Bolufer, P. & Programa de Estudio y Traitmiento de las Hemopatías Malignas. (2004) Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. *Blood*, **103,** 1237–1243.

Sanz, M.A., Tallman, M.S. & Lo-Coco, F. (2005) Practice points, consensus, and controversial issues in the management of patients with newly diagnosed acute promyelocytic leukemia. *Oncologist*, **10,** 806–814.

Sanz, M.A., Montesinos, P., Vellenga, E., Rayón, C., de la Serna, J., Parody, R., Bergua, J.M., León, A., Negri, S., González, M., Rivas, C., Esteve, J., Milone, G., González, J.D., Amutio, E., Brunet, S., García-Laraña, J., Colomer, D., Calasanz, M.J., Chillón, C., Barragán, E., Bolufer, P. & Lowenberg, B. (2008) Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline monochemotherapy: long-term outcome of the LPA 99 multicenter study by the PETHEMA Group. *Blood*, **112,** 3130–3134.

Sanz, M.A., Grimwade, D., Tallman, M.S., Lowenberg, B., Fenaux, P., Estey, E.H., Naoe, T., Lengfelder, E., Büchner, T., Döhner, H., Burnett, A.K. & Lo-Coco, F. (2009) Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European Leukemia Net. *Blood*, **113,** 1875–1891.

Sanz, M.A., Montesinos, P., Rayón, C., Holowiecka, A., de la Serna, J., Milone, G., de Lisa, E., Brunet, S., Rubio, V., Ribera, J.M., Rivas, C., Krsnik, I., Bergua, J., González, J., Díaz-Mediavilla, J., Rojas, R., Manso, F., Ossenkoppele, G., González, J.D. & Lowenberg, B. (2010) Risk-adapted treatment of acute promyelocytic leukemia based on all-trans retinoic acid and anthracycline with addition of cytarabine in consolidation therapy for high-risk patients: further improvements in treatment outcome. *Blood*, **115,** 5137–5146.

Sorensen, K., Levitt, G., Bull, C., Chessells, J. & Sullivan, I. (1997) Anthracycline dose in childhood acute lymphoblastic leukemia: issues of early survival versus late cardiotoxicity. *Journal of Clinical Oncology*, **15,** 61–68.

Specchia, G., Lo Coco, F., Vignetti, M., Avvisati, G., Fazi, P., Albano, F., Di Raimondo, F., Martino, B., Ferrara, F., Selleri, C., Liso, V. & Mandelli, F. (2001) Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not with all-trans retinoic acid: a report by the Gruppo Italiano Malattie Ematologiche dell'Adulto. *Journal of Clinical Oncology*, **19,** 4023–4028.

Suzuki, H., Imaizumi, M., Sato, A., Yoshinari, M., Rikiishi, T., Endo, M., Takano, T., Shimizu, T., Hatae, Y., Fujimoto, T., Hayashi, Y. & Iinuma, K. (2001) Monitoring of minimal residual disease in children with acute promyelocytic leukemia by RT-PCR detecting PML/RARalpha chimeric gene: a retrospective study of clinical feasibility. *Tohoku Journal of Experimental Medicine*, **193,** 127–139.

Tallman, M.S., Nabhan, C., Feusner, J.H. & Rowe, J.M. (2002) Acute promyelocytic leukemia: evolving therapeutic strategies. *Blood*, **99,** 759–767.

Testi, A.M., Biondi, A., Lo Coco, F., Moleti, M.L., Giona, F., Vignetti, M., Menna, G., Locatelli, F., Pession, A., Barisone, E., De Rossi, G., Diverio, D., Micalizzi, C., Aricò, M., Basso, G., Foa, R. & Mandelli, F. (2005) GIMEMA-AIEOPAIDA protocol for the treatment of newly diagnosed acute promyelocytic leukemia (APL) in children. *Blood*, **106,** 447–453.

Warrell, Jr, R.P. (1986) Aclacinomycin A. clinical development of a novel anthracycline antibiotic in the haematological cancers. *Drugs Under Experimental and Clinical Research*, **12,** 275–282.

Zhang, L., Zhao, H., Zhu, X., Chen, Y., Zou, Y. & Chen, X. (2008) Retrospective analysis of 65 Chinese children with acute promyelocytic leukemia: a single center experience. *Pediatric Blood & Cancer*, **51,** 210–215.

Zhou, J., Zhang, Y., Li, J., Li, X., Hou, J., Zhao, Y., Liu, X., Han, X., Hu, L., Wang, S., Zhao, Y., Zhang, Y., Fan, S., Lu, C., Li, L. & Zhu, L. (2010) Single-agent arsenic trioxide in the treatment of children with newly diagnosed acute promyelocytic leukemia. *Blood*, **115,** 1697–1702.

Zunino, F. & Capranico, G. (1990) DNA topoisomerase II as the primary target of anti-tumor anthracyclines. *Anti-Cancer Drug Design*, **5,** 307–317.

Continuous and High-Dose Cytarabine Combined Chemotherapy in Children with Down Syndrome and Acute Myeloid leukemia: Report from the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) AML 9805 Down Study

Takashi Taga, MD,[1]* Yasuto Shimomura, MD,[2] Yasuo Horikoshi, MD,[3] Atsushi Ogawa, MD,[4] Masaki Itoh, MD,[5] Masahiko Okada, MD,[6] Junichi Ueyama, MD,[7] Takeshi Higa, MD,[8] Arata Watanabe, MD,[9] Hirokazu Kanegane, MD,[10] Asayuki Iwai, MD,[11] Yutaka Saiwakawa, MD,[12] Kazuhiro Kogawa, MD,[13] Junko Yamanaka, MD,[14] and Masahito Tsurusawa[2,15]

Background. The aim of the JCCLSG AML 9805 Down study was to evaluate the effect of continuous and high-dose cytarabine combined chemotherapy on the survival outcome of acute myeloid leukemia (AML) with Down syndrome (DS). ***Procedure.*** From May 1998 to December 2006, DS patients with newly diagnosed AML were enrolled. Remission induction therapy consisted of two courses of pirarubicin, vincristine, and continuous-dose cytarabine (AVC1). The patients who achieved complete remission (CR) after two courses of AVC1 were subsequently treated with mitoxantrone and continuous-dose cytarabine (MC), etoposide and high-dose cytarabine (EC) and pirarubicin, vincristine, and continuous-dose cytarabine (AVC2). ***Results.*** Twenty-four patients were enrolled. All patients were younger than 4 years and diagnosed as having acute megakaryoblastic leukemia. Twenty-one patients achieved CR. Three patients died during remission induction therapy due to serious infection. No toxic deaths were observed during remission. All but one patient maintained CR without serious complications. The 5-year overall and event-free survivals were 87.5% ± 6.8% and 83.1% ± 7.7%, respectively. ***Conclusions.*** Continuous and high-dose cytarabine combined chemotherapy with reduced intensity would be effective in DS children with AML. Pediatr Blood Cancer © 2011 Wiley-Liss, Inc.

Key words: AML; Clinical trials; Down syndrome

INTRODUCTION

Down syndrome (DS) is one of the most common chromosomal abnormalities and is associated with an increased risk of leukemia [1]. The clinical and biological features of acute myeloid leukemia (AML) in DS children are quite different from those in children without DS: younger age, lower white blood cell count, and high incidence of acute megakaryoblastic leukemia [2,3]. Before the 1990s, most patients with AML with DS (AML-DS) received suboptimal therapy, resulting in poor outcomes. In 1992, high rates of event-free survival (EFS) with intensive AML treatment were reported from the pediatric oncology group (POG) [4]. After recognition of the favorable outcome of AML-DS patients treated with the AML protocol, recruitment to collaborative studies for AML-DS patients increased, but it became apparent that treatment-related toxicity was high in most series [5–7]. Since then, several collaborative groups have adapted their AML protocols for AML-DS by reducing the dosage of chemotherapeutic agents [6].

We report herein the results of the Japanese Children's Cancer and Leukemia Study Group AML 9805 Down study, which evaluated the feasibility, efficacy, and safety of continuous and high-dose cytarabine combined chemotherapy, which was adapted for DS patients by reducing dose intensity.

PATIENTS AND METHODS

Patients

Between May 1998 and December 2006, 24 AML patients with DS entered the Japanese Children's Cancer and Leukemia Study Group AML 9805 Down study after informed consent was obtained. Neonates with transient myeloproliferative disorder (TMD), defined as appearance of myeloid blasts within the first months of life, and those with spontaneous remission were not included. All children and adolescents less than 18 years of age with no prior treatment were eligible. The initial diagnosis of AML and its subtypes was determined according to the FAB classification by institution pathologists, with central review for most cases.

DOI 10.1002/pbc.22943
Published online in Wiley Online Library (wileyonlinelibrary.com).

Therapy

The scheme of treatment for the JCCLSG AML 9805 Down study is shown in Table I. Remission induction therapy consisted of two courses of AVC1 (cytarabine (Ara-C) 100 mg/m^2/day continuous infusion on days 1–7, pirarubicin 25 mg/m^2 by 60 min infusion on days 2, and 4, and vincristine (VCR) 0.7 mg/m^2 on day 7).

Patients who achieved complete remission (CR) after two courses of AVC1 were subsequently treated with MC (Ara-C 100 mg/m^2/day continuous infusion on days 1–5 and mitoxantrone (MIT) 3.5 mg/m^2 by 60 min infusion days 2–4), EC (high-dose Ara-C 1 g/m^2 every 12 hr on days 1–5, and etoposide 66 mg/m^2 by 2 h infusion on days 2–4) and AVC2 (Ara-C 100 mg/m^2/day continuous infusion on days 1–5, pirarubicin 35 mg/m^2 by 60 min infusion on day 2, and VCR 0.7 mg/m^2 on day 5).

[1]Department of Pediatrics, Shiga University of Medical Science, Japan; [2]Department of Pediatrics, Aichi Medical College, Japan; [3]Department of Hematology and Oncology, Shizuoka Children's Hospital, Japan; [4]Department of Pediatrics, Niigata Cancer Center, Japan; [5]Department of Pediatrics, Fukushima Medical College, Japan; [6]Department of Pediatrics, Nagasaki University, Japan; [7]Department of Pediatrics, Tottori University, Japan; [8]Department of Pediatrics, Ryukyu University, Japan; [9]Department of Pediatrics, Nakadori General Hospital, Japan; [10]Department of Pediatrics, Toyama University, Japan; [11]Department of Hematology and Oncology, Kagawa Children's Hospital, Japan; [12]Department of Pediatrics, Kanazawa Medical College, Japan; [13]Department of Pediatrics, National Defense Medical College, Japan; [14]Department of Pediatrics, International Medical Center of Japan, Japan; [15]Chairperson of JCCLSG, Japan

Conflict of Interest: Nothing to report.

*Correspondence to: Takashi Taga, MD, Department of Pediatrics, Shiga University of Medical Science, Tsukinowa-cho, Seta, Ohtsu, Shiga 520-2192. E-mail: ttaga@belle.shiga-med.ac.jp

Received 2 September 2010; Accepted 5 November 2010

TABLE I. Treatment Regimen of the JCCLSG AML9805 Down Study

	Regimen	Administration	Daily dose	Days
Induction				
AVC1	Cytarabine	IV (24 h)	100 mg/m^2	1–7
	Pirarubicin	IV (1 h)	25 mg/m^2	2–4
	Vincristine	IV	0.7 mg/m^2	7
	Methotrexate	IT	Age-adjusted[a]	1
	Cytarabine	IT	Age-adjusted[a]	1, (5, 10)[b]
	Hydrocortisone	IT	Age-adjusted[a]	1, (5, 10)[b]
Consolidation				
MC	Cytarabine	IV (24 h)	100 mg/m^2	1–5
	Mitoxantrone	IV (1 h)	3.5 mg/m^2	2–4
EC	Cytarabine	IV (2 h)	1 g × 2 /m^2	1–5
	Etoposide	IV (2 h)	66 mg/m^2	2–4
AVC2	Cytarabine	IV (24 h)	100 mg/m^2	1–5
	Pirarubicin	IV (1 h)	35 mg/m^2	2
	Vincristine	IV	0.7 mg/m^2	5
	Methotrexate	IT	Age-adjusted[a]	1
	Cytarabine	IT	Age-adjusted[a]	1
	Hydrocortisone	IT	Age-adjusted[a]	1

Recommended interval of each cycle was 4 weeks. [a]The doses were adjusted according to patient's age as follows: younger than 1 year, methotrexate (MTX) 5 mg, cytarabine (Ara-C) 10 mg, hydrocortisone (HDC) 10 mg; younger than 2 years, MTX 8 mg, Ara-C 20 mg, HDC 15 mg; younger than 3 years, MTX 10 mg, Ara-C 30 mg, HDC 20 mg; 3 years and older, MTX 12 mg, Ara-C 40 mg, HDC 25 mg. [b]For CNS-positive patients. The doses were adjusted according to patient's age as follows: younger than 1 year, cytarabine (Ara-C) 20 mg, hydrocortisone (HDC) 10 mg; younger than 2 years, Ara-C 30 mg, HDC 15 mg; younger than 3 years old, Ara-C 50 mg, HDC 20 mg; 3 years and older, Ara-C 70 mg, HDC 25 mg.

Prophylactic treatment for central nervous system (CNS) leukemia was performed by intrathecal injection of Ara-C, methotrexate, and hydrocortisone on the first day of AVC1 and AVC2. An absolute neutrophil count of more than 1,500/μL and a platelet count of more than 75,000/μL were the criteria for starting the first course of consolidation therapy, and an absolute neutrophil count of more than 1,500/μL and a platelet count of more than 100,000/μL were the criteria for starting the second course.

Definitions and Statistics

Evaluation of each treatment was performed on the 28th day. Treatment response was defined as follows: CR, less than 5% blasts in the bone marrow; partial remission (PR), less than 15% blasts; and no response (NR), more than 15% blasts or progressive disease at other sites.

CNS involvement was diagnosed if more than 5 leukocytes/μL were identified in the cerebrospinal fluid (CSF) in combination with detectable leukemic cells in the cytospin and/or with neurological symptoms (e.g., cranial nerve palsy).

EFS was calculated from the date of the first day of chemotherapy to last follow-up or to the first event (early death, resistant leukemia, relapse, or death from any cause). The EFS time of patients with an induction failure was calculated as zero. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 3.

Univariate comparisons of the survival data were performed using the log-rank test. The Statistical Analysis Software (SAS) computer program was used for the analysis. Follow-up data were actualized as of July 31, 2009.

RESULTS

Patient Characteristics

The relevant initial clinical and hematological data of the 24 patients in this study are shown in Table II. Males predominated, and all patients were younger than 4 years (median age, 17 months). The median white blood cell count was 6,500/μL (range 500–70,900/μL). All patients showed FAB M7 morphologically. No patients had CNS involvement. One patient had an extramedullary mass (skin) at initial diagnosis. Cytogenetic analysis of leukemic blasts was available for 22 patients. Favorable cytogenetics, such as inv (16) and t (8; 21), were not observed. Six patients had normal karyotypes with constitutional trisomy 21 only. The remainder had complex karyotypes with aneuploidy and translocation. GATA1 mutation was confirmed only in one patient.

Seven patients had a history of TMD. No patients of them received cytarabine therapy. Nine patients had documented congenital heart disease. Most patients had either surgically repaired defects or asymptomatic atrial septal defect or ventricular septal defect with normal function.

Overall Outcome

Overall, 21 (87.5%) of 24 patients achieved first remission. One patient relapsed with an isolated extramedullary mass after cessation of chemotherapy. The patient has been in third remission after chemotherapy, electron beam irradiation and cord blood cell transplantation following reduced intensity conditioning. The other 20 patients remain in first CR. Estimated 5-year OS and EFS were 87.5% ± 6.8% and 82.6% ± 7.9%, respectively (Fig. 1). No patients with secondary malignancy and severe cardiotoxicity were observed. Median follow-up period for all patients was 75 (range, 0–131) months.

Treatment-Related Mortality

Three deaths occurred that were not related to leukemia during induction therapy. Two of them occurred during the initial induction therapy, and the other occurred during second induction therapy.

TABLE II. Patients' Characteristic in the JCCLSG AML 9805 Down Study (N = 24)

Characteristic	No	%
Age, months		
Median	17	—
0–12	4	17
12–24	12	46
24–36	4	17
36–48	4	17
Sex		
Male	19	79
Female	5	21
History of TMD		
Yes	7	29
No	13	54
Unknown	4	17
Hepatomegaly		
Yes	10	42
No	12	50
Unknown	2	8
Splenomegaly		
Yes	10	42
No	12	50
Unknown	2	8
WBC, $\times 10^9$/L		
Median	6.5	—
Range	2.8–70.9	—
Hb, g/dL		
Median	8.1	—
Range	3.2–11.8	—
Plt, $\times 10^9$/L		
Median	26	—
Range	3–139	—
Cytogenetics		
Trisomy 8	5	21
Monosomy 7	4	17
Additional 21	2	8

Toxic Events

The incidence of grade 3 or 4 toxicity during induction and each intensification phase of therapy is shown in Table III. Three patients died during remission-induction therapy. One death was attributable to intracranial hemorrhage with disseminated intravascular coagulation, and the others were due to sepsis. The rate of induction death was 12.5%. No toxic deaths were observed during remission.

Prognostic Factors

Extramedullary invasion at initial diagnosis was a significant prognostic factor for 5-year EFS on univariate analysis ($P = 0.046$). Other factors, including sex, initial age, initial WBC, history of TMD, and chromosomal abnormality, were not significant.

DISCUSSION

The results of the JCCSLG AML 9805 Down study, which was conducted to evaluate the efficacy and safety of continuous and high-dose cytarabine combined chemotherapy with reduced intensity for AML-DS patients were presented. All patients enrolled in our study were younger than 4 years and had a phenotype of acute megakaryocytic leukemia (AMKL), which was consistent with previous reports for AML-DS. The number of patients was limited, but this regimen appears to be highly effective because there were no non-responders, and only one patient relapsed.

Contemporary clinical trials for AML-DS children are summarized in Table IV [5–11]. Treatment strategies for AML-DS are based on reduced intensity for AML non-DS, such as BFM and our study, or on a specifically designed strategy, such as the AT/DS study and the AML99 Down study in Japan [8,9]. The EFS of these studies, including the present study, has been between 80% and 90%.

The key drugs for the treatment of AML-DS are anthracyclines, cytarabine, and etoposide; it was also confirmed by in vitro studies that AMKL-DS blasts were significantly more sensitive to these drugs than non-DS AML cells [12]. AMKL-DS blasts are especially sensitive to cytarabine, possibly to the effect of the GATA1 mutations and trisomy 21 on the levels of cytarabine-metabolizing enzymes [13].

In the BFM 98 DS study, with a 3-year EFS of 89%, high-dose cytarabine (3 g/m^2) was used as intensification [6]. The authors reported that a high cure rate could be achieved in DS patients with therapy protocols including high-dose cytarabine. However, they also mentioned that it should be confirmed whether a dosage of 3 g/m^2 of cytarabine is necessary because of its toxicity. In

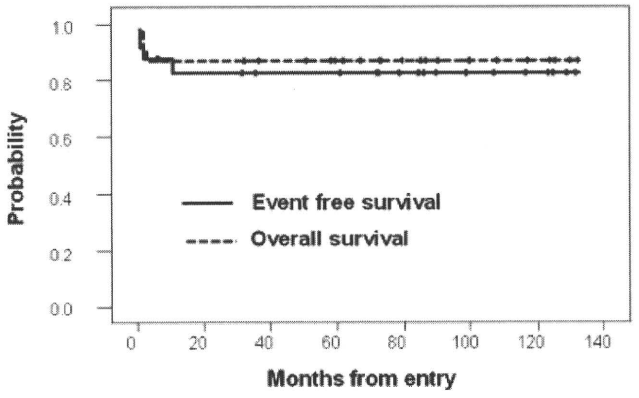

Fig. 1. Actuarial survival rate for the JCCLSG AML 9805 Down study. Of the 24 patients, 22 achieved CR. One patient relapsed. Two patients died during induction therapy. One patient died as a result of sepsis during the first CR. The 5-year overall survival (OS) was 87.5%, and the 5-year EFS was 82.6%.

TABLE III. Severe Adverse Events in the JCCLSG AML9805 Down Study (Grade III–IV)

Adverse events	AVC1-1 no.	n = 24 (%)	AVC1-2 no.	n = 22 (%)	MC no.	n = 21 (%)	EC no.	n = 21 (%)	AVC2 no.	n = 21 (%)
ALT/AST	5	23	2	9	0	0	1	5	0	0
Gastrointestinal	9	41	5	23	5	24	2	10	2	10
Renal	0	0	0	0	0	0	0	0	0	0
Cardiac	0	0	0	0	0	0	0	0	0	0
Pulmonary	1	5	0	0	0	0	0	0	0	0
Neurology	0	0	0	0	0	0	0	0	0	0
Pain	1	5	1	5	0	0	0	0	1	5
Fever/infection	14 (2)	64	9 (1)	41	11	52	15	71	7	33
Others	0	0	0	0	0	0	0	0	0	0

Number of patients who died.

our JCCLSG AML9805 Down study, 1 g/m^2 of cytarabine with etoposide was used for intensification. Serious non-hematological adverse effects, including infection, were not more frequent in this phase than in the other phase of this study (Table III). The dosage of 1 g/m^2 used in the present study may be sufficient for the treatment of AML-DS.

In the Japanese trial AML 99 Down study, the 4-year EFS was 83%, and treatment-related mortality was only 1.4%, which is much lower than that of recent reports for AML-DS [9]. However, relapse and induction failure were more frequent than in other reports with an intensive regimen. The regimen consisted of simple repeating of intermediate doses of pirarubicin and etoposide, so it is possible to reduce the rate of relapse and resistant disease using continuous and high-dose cytarabine combined chemotherapy, as in the JCCLSG AML9805 Down study.

As for other types of leukemia, risk-oriented therapy is proposed if any prognostic factors are identified in AML-DS. In the CCG 2891 study, patients with AML-DS who were older than 2 years had an increased risk of relapse [5]. However, in the BFM 98 DS study and in the Japanese AML 99 Down study, there was no difference in outcome between those 2 years or younger and those older than 2 years [6,9]. The present study also did not identify age older than 2 years as a risk factor, because all 7 patients older than 2 years survived without relapse after completing this protocol.

For cytogenetic factors, monosomy 7 is known to be a risk factor in children with AML [14,15]. In AML-DS, the presence of monosomy 7 adversely affected the outcome in the previous two Japanese trials, but not in the CCG 2891 study [5,8,9]. In the present study, four patients were found to have monosomy 7, and they all maintained remission. Continuous and high-dose cytarabine combined chemotherapy might affect intensification, which negates risk factors such as age and monosomy 7.

It is important to note that only one patient relapsed in the present study. Moreover, the cumulative doses of anthracycline and etoposide in this JCCLSG AML9805 Down study were lower than in other recent reports with intensive regimens for AML-DS. No patients had developed secondary cancer or cardiac insufficiency at the time of this analysis. The survival of DS patients has become longer, and it would be more important to decrease the late toxicity by reducing the cumulative doses of antileukemic drugs for AML-DS patients.

On the contrary, treatment-related mortality occurred in 3 of 24 patients (12.5%), which is more frequent than in other recent reports with intensive regimens for AML-DS. All three patients died from infection during the initial and second courses of this protocol. We could not identify any risk factors for toxicity in these patients, such as age or cardiac disease, compared with the patients who were successfully treated by this protocol. Serious non-hematological adverse effects, including infection, were more frequent during the remission induction phase than during the intensification phase. Induction therapy with combined continuous cytarabine might be toxic for AML-DS patients, although the induction rate is high. On the other hand, toxicity during the intensification phase including high-dose cytarabine was tolerable.

On the basis of the results of the previous Japanese trials and the present study, we have designed a risk-oriented therapy protocol for our next trial with AML-DS. Patients with M2, M3 marrow after induction therapy by pirarubicin, intermediate-dose cytarabine, and etoposide classified into a high-risk group will receive the continuous and high-dose cytarabine combined regimen of this JCCSLG AML9805 Down study.

TABLE IV. Comparison of Recent Clinical Trials for AML-DS

Study	Registry (year)	N	Daunorubicin (mg/m^2)	Ara-C (mg/m^2)	Etoposide (mg/m^2)	TRM (%)	OS (%)	EFS (%)
BFM98 for DS	1998–2003	67	220–240	23–29,000	950	5	91	89 (3y)
BFM93	NA	51	220–400	23,000	950	4	70	68 (3y)
NOPHO AML93	1988–2002	41	300	48,600	1,600	5	NA	85 (8y)
MRC AML10/12	1988–2002	46	670	10,600	NA	15	74	74 (5y)
CCG 2861/2891	1989–1999	160	320	15,800	1,600	4	79	77 (6y)
POG 9421	1995–1999	57	100	20,700	—	0	NA	79 (3y)
AT/Down	1987–1997	33	100–400	4,200	2,700	9	NA	80 (8y)
AML99 DS	2000–2004	72	250	3,500	2,250	1	84	83 (4y)
JCCLSG 9805DS	1998–2006	24	190	12,600	200	12.5	88	83 (5y)

TRM, treatment-related mortality; OS, overall survival; EFS, event-free survival; NA, not evaluated.

ACKNOWLEDGMENT

We are grateful to all participating institutions in the JCCLSG and all members of the JCCLSG AML committee for their contributions to the thorough follow-up and data collection in each case.

REFERENCES

1. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet 2000;355:165–169.
2. Kojima S, Matsuyama T, Sato T, et al. Down's syndrome and acute leukemia in children: an analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. Blood 1990;76:2348–2353.
3. Zipursky A, Thorner P, De Harven E, et al. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. Leukemia Res 1994;18:163–171.
4. Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia in Down's syndrome in highly responsive to chemotherapy: experience on Pediatric Oncology Group AML study 8498. Blood 1992;80:2210–2214.
5. Gamis AS, Woods WG, Alonzo TA, et al. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. J Clin Oncol 2003; 21:3415–3422.
6. Creutzig U, Reinhardt D, Diekamp S, et al. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia 2005;19:1355–1360.
7. Rao A, Hills RK, Stiller C, et al. Treatment for myeloid leukaemia of down syndrome: population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. Br J Haematol 2006;132:576–583.
8. Kojima S, Sako M, Kato K, et al. An effective chemotherapy regimen for acute myeloid leukemia and myelodysplastic syndrome with Down's syndrome. Leukemia 2000;14:786–791.
9. Kudo K, Kojima S, Tabuchi K, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol 2007;25:5442–5447.
10. Abildgaard L, Ellebaek E, Gustafsson G, et al. Optimal treatment intensity in children with Down syndrome eloid leukaemia: data from 56 children treated on NOPHO-AML protocols and review of the literature. Ann Haematol 2006;85:275–280.
11. Stevens RF, Hann IM, Wheatley K, et al. Marked improvements in outcome with chemotherapy alone in paedriac acute myeloid leukemia: results of the United Kingdom medical research council's 10th AML trial. MRC childhood leukaemia working party. Br J Haematol 1998;101:130–140.
12. Zwaan CM, Kaspers GJ, Pieters R, et al. Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. Blood 2002;99:245–251.
13. Ge Y, Stout ML, Tatman DA, et al. GATA1, cytidine deaminase, and the high cure rate of Down syndrome children with acute megakaryocytic leumemia. J Natl Cancer Inst 2005;97:226–231.
14. Raimondi SC, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative Pediatric Oncology Group study-POG8821. Blood 1999;94:3707–3716.
15. Wells RJ, Arthur DC, Srivastava A, et al. Prognostic variables in newly diagnosed children and adolescents with acute myeloid leukemia: Children's Cancer Group Study 213. Leukemia 2002; 16:601–607.

Int J Hematol (2011) 93:135–137
DOI 10.1007/s12185-010-0754-y

LETTER TO THE EDITOR

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

Hideaki Ohta · Shotaro Iwamoto · Nobutaka Kiyokawa · Masahito Tsurusawa · Takao Deguchi · Kozo Takase · Junichiro Fujimoto · Keizo Horibe · Yoshihiro Komada

Received: 6 August 2010 / Revised: 5 December 2010 / Accepted: 14 December 2010 / Published online: 5 January 2011

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

For the Immunological Diagnosis Committee of the Japanese Pediatric Leukemia/Lymphoma Study Group.

H. Ohta (✉)
Department of Pediatrics,
Osaka University Graduate School of Medicine,
Yamadaoka 2-2, Suita, Osaka 565-0871, Japan
e-mail: ohta@ped.med.osaka-u.ac.jp

S. Iwamoto · T. Deguchi · Y. Komada
Department of Pediatrics and Developmental Science,
Mie University Graduate School of Medicine,
Tsu, Mie, Japan

N. Kiyokawa
Department of Developmental Biology,
National Center for Child Health and Development,
Setagaya-ku, Tokyo, Japan

M. Tsurusawa
Department of Pediatrics, Aichi Medical University,
Nagakute, Aichi, Japan

K. Takase
Department of Health Science Policies,
Division of Research Development,
Graduate School of Medical and Dental Sciences,
Tokyo Medical and Dental University,
Bunkyo-ku, Tokyo, Japan

J. Fujimoto
Center for Clinical Research, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan

K. Horibe
Clinical Research Center, National Hospital Organization
Nagoya Medical Center, Nagoya, Aichi, Japan

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

72.4 and 58.5%, respectively. All cases expressed CD41 and/or CD42b. CD36 was expressed at a high frequency, but its expression was also seen in other subtypes (M4, M5, and M6). Myeloid antigens (CD13 and CD33) were expressed in most cases, but lack of MPO expression was observed. Hematopoietic progenitor-associated antigens (CD34 and CD117) were expressed in many cases, and CD7 was expressed in 69.6% of cases.

In conclusion, each subtype of AML possesses distinguishing features of antigen expression. Some antigens appear to be associated with certain subtypes, but are not necessarily specific. Uncommon expression must be interpreted in the context of the entire immunophenotyping profile for correct identification of AML subtypes.

Acknowledgment This study was supported by a grant for Clinical Cancer Research from the Ministry of Health, Labour, and Welfare of Japan.

References

1. Bene MC, Bernier M, Casasnovas RO, Castoldi G, Doekharan D, van der Holt B, et al. Acute myeloid leukaemia M0: haematological, immunophenotypic and cytogenetic characteristics and their prognostic significance: an analysis in 241 patients. Br J Haematol. 2001;113:737–45.
2. Behm FG. Diagnosis of childhood acute myeloid leukemia. Clin Lab Med. 1999;19:187–237. vii.
3. Kotylo PK, Seo IS, Smith FO, Heerema NA, Fineberg NS, Miller K, et al. Flow cytometric immunophenotypic characterization of pediatric and adult minimally differentiated acute myeloid leukemia (AML-M0). Am J Clin Pathol. 2000;113:193–200.
4. Creutzig U, Harbott J, Sperling C, Ritter J, Zimmermann M, Loffler H, et al. Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87. Blood. 1995;86:3097–108.
5. Kaleem Z, Crawford E, Pathan MH, Jasper L, Covinsky MA, Johnson LR, et al. Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. Arch Pathol Lab Med. 2003;127:42–8.
6. Chabannon C, Wood P, Torok-Storb B. Expression of CD7 on normal human myeloid progenitors. J Immunol. 1992; 149:2110–3.
7. Oshimi K. Progress in understanding and managing natural killer-cell malignancies. Br J Haematol. 2007;139:532–44.
8. Kita K, Nakase K, Miwa H, Masuya M, Nishii K, Morita N, et al. Phenotypical characteristics of acute myelocytic leukemia associated with the t(8;21)(q22;q22) chromosomal abnormality: frequent expression of immature B-cell antigen CD19 together with stem cell antigen CD34. Blood. 1992;80:470–7.
9. Hurwitz CA, Raimondi SC, Head D, Krance R, Mirro J Jr, Kalwinsky DK, et al. Distinctive immunophenotypic features of t(8;21)(q22;q22) acute myeloblastic leukemia in children. Blood. 1992;80:3182–8.
10. Campana D, Behm FG. Immunophenotyping of leukemia. J Immunol Methods. 2000;243:59–75.

ASBMT
American Society for Blood and Marrow Transplantation

Prognostic Factors for Outcomes of Pediatric Patients with Refractory or Relapsed Acute Leukemia Undergoing Allogeneic Progenitor Cell Transplantation

Nobuhiro Watanabe,[1] Yoshiyuki Takahashi,[2] Kimikazu Matsumoto,[1] Asahito Hama,[2] Hideki Muramatsu,[2] Sayoko Doisaki,[2] Keizo Horibe,[3] Koji Kato,[1] Seiji Kojima[2]

Allogeneic stem cell transplantation (SCT) is the only curative therapy for patients with refractory or relapsed acute leukemia, although the prognosis remains poor. Few reports have described outcomes of SCT in pediatric patients with refractory acute leukemia. To identify prognostic factors for these patients, we retrospectively evaluated SCT outcomes for advanced acute leukemia in 82 pediatric patients from 3 transplant units in Nagoya City between 1990 and 2008. Median age at transplantation was 8 years (range, 0.5-17 years). Transplantation was performed in the first refractory relapse for 53 patients (64.6%), in the second or subsequent relapse for 16 patients (19.5%), and during primary induction failure for 13 patients (15.9%). Only 4 patients (4.9%) underwent transplantation in the untreated first relapse, and 39 patients (47.6%) received unrelated donor progenitor cells. Of the 82 patients, 61 died (77.9%), with a median survival of 7.1 months (95% confidence interval [CI], 4.2-10.0 months). Median disease-free survival (DFS) was 4.7 months (95% CI, 2.6-6.9 months). In multivariate analysis, peripheral blood blasts, cord blood transplantation, and more than 3 courses of previous salvage chemotherapy were predictive of DFS. These results support the notion that allogeneic SCT offers only a small chance of cure for most pediatric patients with refractory or relapsed acute leukemia, and suggest that reduction of the leukemia burden and earlier optimal timing of transplantation are essential for long-term survival even in patients with refractory acute leukemia.

Biol Blood Marrow Transplant 17: 516-523 (2011) © *2011 American Society for Blood and Marrow Transplantation*

KEY WORDS: Allografting, Pediatric patients, Acute leukemia, Refractory or relapsed

INTRODUCTION

Although advances in chemotherapy have improved the prognosis for patients with acute leukemia, outcomes remain poor in patients with refractory or relapsed disease [1-7]. Moreover, much of the literature has reported results for pediatric and adult patients together, making outcomes in pediatric patients alone difficult to determine. The present study sought to identify prognostic factors influencing outcomes after stem cell transplantation (SCT) in children with acute leukemia who had not achieved remission with chemotherapy.

From the [1]Division of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [2]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; and [3]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan.

Financial disclosure: See Acknowledgments on page 522.

Correspondence and reprint requests: Nobuhiro Watanabe, MD, Division of Hematology and Oncology, Shizuoka Children's Hospital of Shizuoka Prefecture, 860 Urushiyama, Aoi-ku, Shizuoka 420-8660, Japan (e-mail: nobuhiro.watanabe@sch.pref.shizuoka.jp).

Received May 17, 2010; accepted July 21, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.07.019

PATIENTS AND METHODS

Three transplant units in Nagoya City were asked to provide data on all pediatric patients (aged ≤17 years at the time of transplantation) who underwent allogeneic SCT for acute lymphoblastic leukemia (ALL; n = 48), acute myelogenous leukemia (AML; n = 31), or acute undifferentiated leukemia (AUL; n = 3) after failing to achieve remission between 1990 and October 2008. Remission was defined as morphologically normal bone marrow (BM) without cytogenetic evidence of leukemia, and a morphologically normal peripheral blood (PB) smear with recovery of PB hematologic values, including a platelet count $>100 \times 10^9$/L and an absolute neutrophil count $>1.5 \times 10^9$/L. Chromosomal abnormalities classified as good prognostic features included AML with translocation 8;21 (n = 3) and ALL with hyperdiploid karyotype (n = 2). Karyotypes considered to have a poor prognosis included AML and ALL with abnormalities of chromosome 7

(n = 4), those involving the long arm of chromosome 11 (n = 12), and translocation 1;19 (n = 2), t4;11 (n = 4), or t9;22 (n = 10). The other 46 patients (including 14 patients with normal karyotype) were assigned to the intermediate-risk category.

The results of HLA testing performed using standard serologic methods for HLA-A, -B, -DR, and -DR identity were confirmed by high-resolution molecular typing. The conditioning regimens used for transplantation and protocols for graft-versus-host disease (GVHD) prophylaxis were those used at our institutions during the period when the patients underwent transplantation. Conditioning regimens were classified as total body irradiation (10-12 Gy, divided into 4 fractions)-based myeloablative (MA), high-dose chemotherapy-based MA, or reduced-intensity (RIC) [8]. Patients and donors provided written informed consent, and unrelated donor cells were procured under the auspices of the Japanese Marrow Donor Program following the applicable current guidelines.

Statistical Analysis

Data were collected to allow study of the following subgroups: age at SCT (≤10 years vs ≥11 years), year of transplantation (1990-2000 vs 2001-2008), disease status (primary induction failure [PIF] vs first or later refractory relapse, untreated relapse, or chemoresistant relapse), type of leukemia (ALL vs AML/AUL), number of salvage treatment courses before transplantation (≤2 vs ≥3), cytogenetics (good vs other), conditioning regimen (total body irradiation vs chemotherapy only, MA vs RIC), donor source (BM/PB vs cord blood [CB]; HLA disparity), duration of previous first complete remission (CR1) (≤1 year vs >1 year), high disease burden (% BM blasts before SCT or presence of circulating blasts), acute GVHD (aGVHD; grade 0-I vs grade II-IV), and chronic GVHD (cGVHD; none vs limited vs extensive). HLA disparity was considered a trinary variable for risk factor analysis. The HLA-higher mismatched group included patients undergoing transplantation from a related donor with ≥2 antigen mismatches, unrelated CB donors with ≥2 antigen mismatches, and unrelated BM donors with ≥1 antigen mismatches. The HLA-middle mismatched group included related donors with 1 antigen mismatch, CB donors with 1 antigen mismatch, and unrelated matched BM donors. The HLA-less mismatched group included HLA-matched related donors.

Unadjusted survival probabilities were estimated using the Kaplan-Meier method. Comparisons of unadjusted between-group survival rates were made using the log-rank test. Univariate analyses were performed for various pretransplantation and transplantation variables related to disease-free survival (DFS), nonrelapse mortality (NRM), and relapse rate at 5 years posttransplantation. Cox proportional hazards regression modeling was used to assess the ability of patient characteristics and treatment-related variables to predict survival. All variables showing a probable association ($P < .10$) with DFS, NRM, or relapse rate in univariate analyses were included in the Cox proportional hazards model. Time-dependent covariates were used to study aGVHD and cGVHD. All statistical tests were two-sided, and differences were considered statistically significant at $P < .05$. Associations between discrete variables were assessed by Fisher's exact and generalized exact tests. All analyses were performed using SPSS software (SPSS, Chicago, IL).

RESULTS

Patient Characteristics

Demographic data and disease characteristics are shown in Table 1. Median patient age at transplantation was 8 years (range, 0.5-17 years). Transplantation was performed during the first refractory relapse in 53 patients (64.6%), during a second or subsequent relapse in 16 patients (19.5%), and during PIF in 13 patients (15.9%). Four patients (4.9%) underwent transplantation during an untreated first relapse, and the remaining 78 patients were unable to attain CR despite induction chemotherapy. Fifty-four patients (65.8%) had received ≥3 courses of salvage chemotherapy, and 49 patients (59.7%) had experienced early relapse after a remission lasting less than 1 year. For the 43 patients who received an allograft from a related donor, 33 (40.2%) were HLA-identical and 10 (12.2%) were HLA-mismatched. Of the 39 unrelated donors, 20 (24.4%) were HLA-identical and 19 (23.1%) were HLA-mismatched. The transplant source was BM in 65 patients (79.3%), mobilized PB stem cells in 9 patients (11.0%), and CB in 8 patients (9.8%). Three patients (3.7%) received an RIC regimen, whereas 79 patients (96.3%) received an MA conditioning regimen. GVHD prophylaxis consisted primarily of tacrolimus combination therapy (37 [45.1%]), cyclosporine combination therapy (21 [25.6%]), or methotrexate alone (21 [25.6%]).

Survival, NRM, and Relapse Rate

Sixty-one of the 82 patients died (77.9%), with a median survival of 7.1 months (95% confidence interval [CI], 4.2-10.0 months). A total of 66 patients (83.4%) either died or displayed disease progression. Median DFS was 4.7 months (95% CI, 2.6-6.9 months) (Figure 1). Median follow-up for the disease-free survivors was 8.6 years (range, 0.37-19 years). On univariate analysis, significant prognostic factors for DFS were stem cell type, presence of PB blasts before SCT, more than 3 courses of previous salvage chemotherapy, and cGVHD (Table 2).

NRM occurred in 25 patients (30.5%), including 9 from respiratory failure, including interstitial

Table 1. Patient Characteristics

	n	%
Number of patients	82	
Sex		
Male	52	63.4
Female	30	36.4
Diagnosis		
ALL	48	58.5
AML	31	37.8
AUL	3	3.7
Age		
Median (range), years	8 (0.5-17)	
≤10 years	55	67.1
≥11 years	27	32.9
Year of transplantation		
1990-2000	44	53.7
2001-2008	38	46.3
Disease status before SCT		
Primary induction failure	13	15.9
First relapse refractory	53	64.6
>First relapse refractory	16	19.5
Number of chemotherapy cycles before SCT		
Untreated first relapse	4	4.9
1 cycles	8	9.8
2 cycles	16	19.5
≥3 cycles	54	65.8
Cytogenetic subgroup		
Good	5	6.1
Intermediate	44	53.7
Bad	30	36.6
Missing	2	2.4
Stem cell type		
BM	65	79.3
PB	9	11
CB	8	9.8
Donor type		
BM/PB		
Matched related	33	40.2
Mismatched related	10	12.2
One locus mismatched	3	3.7
Two loci mismatched	7	8.5
Matched unrelated	20	24.4
Mismatched unrelated		
One locus mismatched	11	13.4
CB	8	9.8
One locus mismatched	3	3.7
Two loci mismatched	5	6.1
Donor sex		
Male	38	46.3
Female	44	53.7
Conditioning regimen		
Reduced-intensity conditioning		
Yes	3	3.7
No	79	96.3
Total body irradiation		
Yes	76	92.7
No	6	7.3
Use of ATG	11	13.4
Use of busulfan	47	57.3
Use of cyclophosphamide	14	17.1
Use of melphalan	66	80.5
GVHD prophylaxis		
MTX alone	21	25.6
Cyclosporine + MTX	19	23.2
Cyclosporine + MTX + PSL	1	1.2
Cyclosporine + PSL	1	1.2
Tacrolimus + MTX	36	43.9
Tacrolimus + MMF	1	1.2
None	3	3.7
Leukemia burden at SCT		
Presence of circulating blasts		
Yes	44	53.7
No	38	46.3
≥25% marrow blasts		
Yes	57	69.5
No	20	24.4
Missing	5	6.1
Duration of CR1, months		
<6	33	40.2
6-12	16	19.5
≥12	31	37.8
Missing	2	2.4

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; AUL, acute undifferentiated leukemia; SCT, stem cell transplantation; BM, bone marrow; PB, peripheral blood; CB, cord blood; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; MTX, methotrexate; PSL, prednisolone.

pneumonia, diffuse alveolar hemorrhage, and bronchiolitis obliterans; 6 from infection; 5 from severe aGVHD; 3 from multiorgan failure, including veno-occlusive disease; and 2 from encephalopathy (Figure 2). On univariate analysis, no risk factors were associated with higher NRM rates.

A total of 41 patients (50%) relapsed, with a median relapse time from SCT of 10.7 months (95% CI, 3.2-18.1 months) (Figure 3). Risk factors associated with relapse rate included stem cell type, presence of PB blasts before SCT, the number of salvage chemotherapy, and cGVHD (Table 3). In contrast, age, sex, diagnosis (ALL vs AML/AUL), cytogenetic subgroup, and duration of CR1 were not significantly associated with clinical outcomes.

Multivariate Analysis

Cox regression analysis showed that the number of treatment courses before SCT, stem cell type, and the presence of PB blasts before SCT were predictive of DFS (Table 2). No prognostic factors for NRM were identified, whereas the number of treatment courses before SCT, stem cell type, and presence of PB blasts were associated with relapse rate (Table 3). In patients receiving a BM or PB graft, HLA disparity also was associated with DFS and relapse rate (Tables 2 and 3).

Figure 4 shows the manner in which the predicted DFS probability under the Cox regression model summarized in Table 2 varies with PB blasts and number of treatment courses before SCT. In the 54 patients receiving ≥3 courses of treatment before transplantation, DFS at 5 years was 28.0% (range, 19.0%-37.0%) in 25 patients without circulating PB blasts and 3.4% (range, 0%-6.8%) in 29 patients with PB blasts ($P = .004$).

DISCUSSION

The recent development and implementation of more aggressive chemotherapy protocols and better

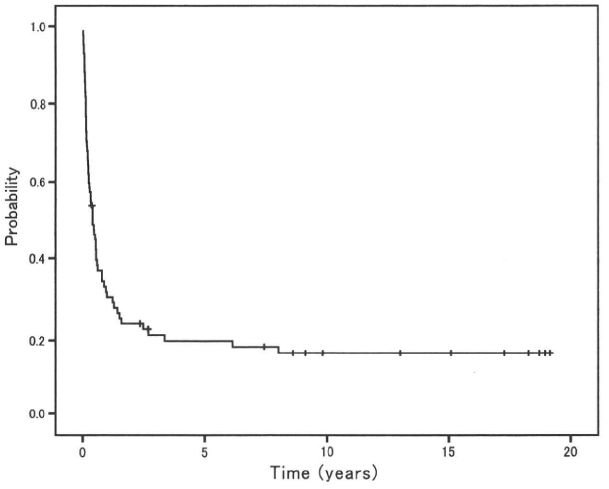

Figure 1. Kaplan-Meier curve for DFS.

supportive care following rigorous clinical trials have led to improving survival rates in children with acute leukemias, including lymphoid and myelogenous malignancies [9-14]. Nonetheless, current therapeutic results remain unsatisfactory, particularly for patients with primary refractory or relapsed disease despite salvage chemotherapy. In patients with refractory or relapsed acute leukemia treated with allogeneic SCT, long-term survival is generally poor [1-7]. Previous studies of outcomes of allogeneic SCT for advanced acute leukemia have been limited by the small number of patients and the inclusion of a range of disease statuses, from remission to refractory or relapsed disease. Most studies also have included both pediatric and adult patients, and few have examined pediatric patients with acute leukemia who never achieved CR [4]. In the present study, we evaluated a large number of pretreatment variables for effects on transplantation outcomes in pediatric patients. We found that the number of treatment courses before SCT, stem cell type, and the presence of PB blasts before SCT were independently associated with DFS in pediatric patients with refractory or relapsed acute leukemia, as has been reported for adult patients [4-6,15-18]. In our study, two-thirds of patients had received more than 3 courses of chemotherapy, making this a heavily pretreated

Table 2. Prognostic Factors Associated with DFS

			Multivariate		
Variable	5-Year DFS, %	Univariate *P* Value	HR	95% CI	*P* Value
All recipients					
Year of transplantation					
1990-2000	29.5 ± 6.9	.098			
2001-2008	13.2 ± 5.5				
Number of previous therapies					
≤2	35.7 ± 9.1	.024	1		
≥3	14.8 ± 4.8		3.62	1.62-8.08	.002
Stem cell type					
BM/PB	24.3 ± 5.0	.015	1		
CB	0		5.69	1.35-24.03	.018
% BM blasts before SCT					
<25% (n = 20)	35.0 ± 10.7	.097			
≥25% (n = 57)	19.3 ± 5.2				
PB blast before SCT					
Blast-negative	36.8 ± 7.8	<.001	1		
Blast-positive	9.1 ± 4.3		3.24	1.61-6.54	.001
Chronic GVHD					
None	20.3 ± 6.8	.013			
Limited/extensive	42.3 ± 9.7				
BM/PB recipients					
Year of transplantation					
1990-2000	31.7 ± 7.3	.091			
2001-2008	15.2 ± 6.2				
Number of previous therapies					
≤2	43.5 ± 10.3	.014	1		
≥3	15.7 ± 5.7		4.98	2.02-12.26	<.001
PB blast before SCT					
Blast-negative	38.9 ± 8.1	.004	1		
Blast-positive	10.5 ± 5.0		2.53	1.19-5.41	.016
HLA disparity					
Match	30.2 ± 6.3	.013	1		
Mismatch	9.5 ± 6.4		2.38	1.05-5.35	.037
Chronic GVHD					
None	21.9 ± 7.3	.014			
Limited/extensive	44.0 ± 9.9				

DFS indicates disease-free survival; BM, bone marrow; PB, peripheral blood; CB, cord blood; SCT, stem cell transplantation; GVHD, graft-versus-host disease.

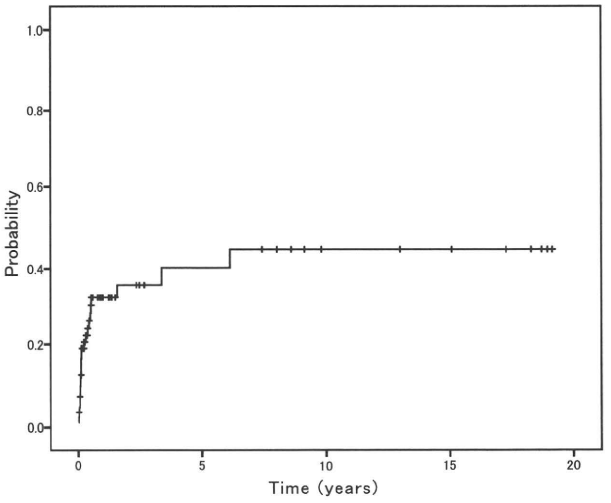

Figure 2. Time to NRM for 82 patients with refractory or relapsed acute leukemia.

group of patients. In multivariate analysis, fewer courses of pretransplantation chemotherapy was associated with better DFS and relapse rate. Similar observations have been reported previously [3,15]. As Schmid et al. [15] reported, this difference is not attributable to a higher NRM, as might be expected given the higher cumulative organ toxicity in heavily pretreated patients. These authors also reported that disease stage at the time of transplantation did not differ between patients pretreated with 2 or ≥3 courses of salvage chemotherapy. These observations might offer clues to guide the selection of chemoresistant leukemic cells during repeated reinduction attempts and possibly favor earlier timing of allogeneic SCT once acute leukemia appears to be refractory to chemotherapy. Prompt transplantation using HLA-mismatched/haploidentical blood and BM grafts might be warranted for patients without a suitable related matched donor [19]. Patients who

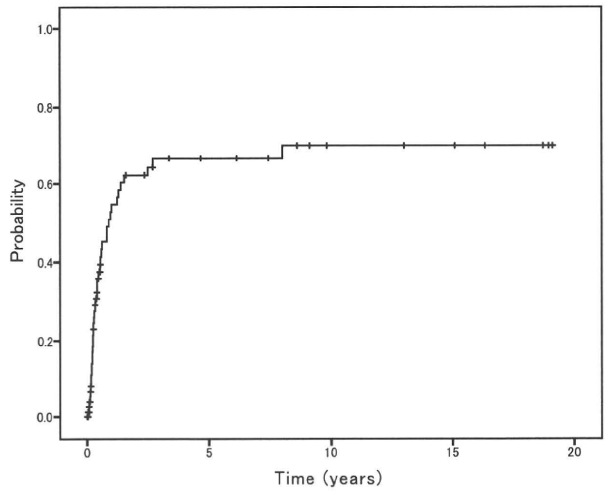

Figure 3. Time to relapse for 82 patients with refractory or relapsed acute leukemia.

do not achieve remission after multiple salvage therapies with circulating PB blasts might not be good candidates for SCT.

We hypothesized that the leukemic burden, as measured by the PB blast count and BM leukemia infiltrate, would be important prognostic factors for outcome. The results of our univariate and multivariate analyses suggest that PB blasts can be predictive of DFS. Our analysis confirms an association between high disease burden and poor survival, as reported previously [16-18]. The high relapse rate after SCT suggests that current high-dose chemoradiation regimens are often inadequate to eradicate leukemia. Multiple preparative regimens have been tested in the phase II setting in an attempt to improve outcomes in patients with advanced leukemia, but none has demonstrated superiority over other regimens [20]. This finding supports the current practice of attempting cytoreduction before proceeding with transplantation in patients showing circulating blasts or extensive BM disease. This argues in favor of further attempts at blast reduction in patients with high blast counts, inevitably increasing the time to transplantation.

Andrew et al. [21] reported an association between a graft-versus-leukemia (GVL) effect and GVHD after allogeneic SCT for refractory or relapsed acute leukemia. However, in our study, cGVHD was significantly associated with DFS and relapse rate in the univariate analysis, but not in multivariate Cox regression model. In the study of Andrew et al., the leukemic burden before transplantation was not included as a pretransplantation variable in the analysis of factors that had a significantly effect on clinical outcomes. A European report of a large number of patients receiving cyclosporine and methotrexate as GVHD prophylaxis documented a greater GVL effect associated with aGVHD in CR1 compared with relapse [22]. High leukemic burden and selection of chemoresistant leukemic cells during repeated reinduction attempts may cause the failure of complete donor chimerism, preventing the development of cGVHD or a GVL effect in these patients.

We found no difference in DFS, NRM, or relapse rate between patients with ALL and those with AML/AUL. A study based on data from the International Bone Marrow Transplant Registry reported that the GVL effect was greatest in AML, was of borderline significance in chronic myelogenous leukemia, and was absent in ALL [23]. However, these patients underwent BM transplantation while in CR1. Ringden et al. [22] noted that the GVL effect is most obvious in early disease. Our findings demonstrate that leukemic burden is a more important factor in DFS than the difference in disease between ALL and AML/AUL in patients receiving SCT with refractory leukemia.

As in previous reports [16,21], we found a nonsignificant trend in survival associated with classical

Table 3. Risk Factors Associated with Relapse Rate

Variables	5-Year Relapse Rate, %	Univariate *P* Value	Multivariate		
			HR	95% CI	*P* Value
All recipients					
Number of previous therapies					
≤2	59.6 ± 12.5	.076	1		
≥3	70.7 ± 7.4		3.01	1.29-7.03	.011
Stem cell type					
BM/PB	67.7 ± 7.0	.034	1		
CB	100		4.78	1.18-19.42	.029
PB blast before SCT					
Blast-negative	53.4 ± 11.0	<.001	1		
Blast-positive	88.2 ± 6.3		3.19	1.55-6.56	.002
Chronic GVHD					
None	78.2 ± 7.4	.004			
Limited/extensive	53.0 ± 12.6				
BM/PB recipients					
Number of previous therapies					
≤2	53.6 ± 13.9	.059	1		
≥3	75.7 ± 7.3		3.66	1.51-8.83	.004
PB blast before SCT					
Blast-negative	51.9 ± 11.2	.002	1		
Blast-positive	86.8 ± 7.0		2.33	1.05-5.16	.038
HLA disparity					
Match	61.5 ± 8.3	.041	1		
Mismatch	83.2 ± 10.5		2.51	1.09-5.77	.031
Chronic GVHD					
None	76.0 ± 8.1	.009			
Limited/extensive	53.1 ± 12.6				

BM indicates bone marrow; PB, peripheral blood; CB, cord blood; SCT, stem cell transplantation; GVHD, graft-versus-host disease.

prognostic cytogenetic features. Other groups have reported a low relapse rate and better long-term survival after SCT in patients with inv16 and t15;17, even in non-complete remission [24,25]. In our study, there were no patients with inv16 and t15;17, and few patients with a good-risk chromosomal abnormality. Given the limited number of patients with good-risk abnormalities, the actual significance of each additional abnormality should be investigated in a larger number of patients.

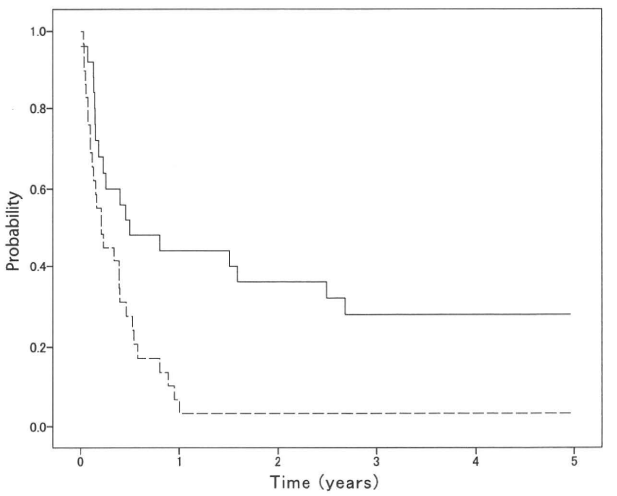

Figure 4. Effect of previous therapy and the presence of PB blasts pre-transplantation on DFS at 5 years. Outcomes for 54 patients who received ≥3 courses of therapy before transplantation are shown. DFS at 5 years was 28.0% (range, 19.0%-37.0%) in 25 patients without circulating PB blasts (solid line) and 3.4% (range, 0%-6.8%) in 34 patients with PB blasts (dashed line) ($P = .004$).

In our study, CB transplantation was significantly associated with DFS and relapse rate. The diminished GVHD after CB transplantation reported by some investigators raises the concern that CB-derived cells might not be capable of generating a sufficient GVL response. However, the incidence of recurrent leukemia after CB transplantation does not differ from that reported in BM or PB transplantation [26]. Because of the limited number of patients in our subgroup analyses and the possibility of an unidentified bias in stem cell source selection, our findings should be verified by further analysis in a larger population.

In our BM and PB recipients, HLA disparity was significantly associated with DFS and relapse. This finding suggests that there may be several reasons for the increased risk of relapse in the HLA-mismatched group, such as selection bias and more heavy immunosuppressive therapy. Increased immunosuppression might decrease the GVL effect [27].

Despite all of the recent advances in SCT, survival of children with refractory or relapsed acute leukemia has not changed significantly since the first reports of BM transplantation more than 3 decades ago [28]. Our results suggest that innovative strategies are justified if allografting is to be contemplated in most pediatric patients with refractory acute leukemia. Recent sequential use of intensive chemotherapy, RIC transplantation, and prophylactic donor lymphocyte