Table 1
Treatment schedules

ALL90 study	ALL93 study	ALL97 study	
Remission induction			
VCR 1.4 mg m^{-2} IV × 3–4	VCR 1.3 mg m^{-2} IV × 4	VCR 1.3 mg m^{-2} IV × 4	
ADR 25 mg m^{-2} IV × 1–3	ADR 30 mg m^{-2} IV × 6	DNR 45 mg m^{-2} IV × 3	
CPM 600 mg m^{-2} IV × 1–2	CPM 600 mg m^{-2} IV × 1	CPM 1200 mg m^{-2} IV × 1	
PSL 40–60 mg m^{-2} PO × 14	PSL 40 mg m^{-2} PO × 10	PSL 60 mg m^{-2} PO × 14	
L-ASP 5000 IU m^{-2} IV or SC × 0–2	L-ASP 6000 IU m^{-2} IV × 7	L-ASP 3000 IU m^{-2} IV × 6	
MIT 6 mg m^{-2} IV × 1–3			
Consolidation			
ETP 100 mg m^{-2} IV × 5	MIT 6 mg m^{-2} IV × 3	C-1	C-5
BHAC 200 mg m^{-2} IV × 5	ETP 100 mg m^{-2} IV × 5	VCR 1.3 mg m^{-2} IV × 1	Same as C-1 except for substituting
VDS 2 mg m^{-2} IV × 1	Ara-C 100 mg m^{-2} IV × 6	ADR 60 mg m^{-2} IV × 1	DEX 10 mg m^{-2} PO × 3 for PSL
PSL 40 mg m^{-2} PO × 5	IT × 1	CPM 1000 mg m^{-2} IV × 1	
IT × 1		PSL 60 mg m^{-2} PO × 3	
	MTX 600 mg m^{-2} IV × 2	IT × 1	C-6
MIT 7 mg m^{-2} IV × 3	L-ASP 10000 IU m^{-2} IV × 2		Same as C-2 except for substituting
BHAC 200 mg m^{-2} IV × 5	IT × 1	C-2	DEX 10 mg m^{-2} PO × 3 for PSL
IT × 1		MTX 500 mg m^{-2} IV × 1	
	ACR 14 mg m^{-2} IV × 8	VCR 1.3 mg m^{-2} IV × 1	
CPM 800 mg m^{-2} IV × 1	Ara-C 70 mg m^{-2} IV × 7	ADR 45 mg m^{-2} IV × 1	C-7
ACR 50 mg m^{-2} IV × 2	PSL 40 mg m^{-2} PO × 7	PSL 60 mg m^{-2} PO × 3	Same as C-5
VDS 2 mg m^{-2} IV × 1	IT × 1	IT × 1	
PSL 40 mg m^{-2} PO × 5			C-8
IT × 1		C-3	Same as C-4 except for substituting
		Same as C-1	MIT 8 mg m^{-2} IV × 2 for ETP
MTX 400 mg m^{-2} IV × 1			
L-ASP 6000 IU m^{-2} IM or SC × 2		C-4	
		ETP 100 mg m^{-2} IV × 4	
		Ara-C 200 mg m^{-2} IV × 4	
		6MP 60 mg m^{-2} IV × 4	
		PSL 60 mg m^{-2} PO × 4	
		IT × 1	
Intensification			
DNR 30 mg m^{-2} IV × 3	ADR 30 mg m^{-2} IV × 6		
VDS 2 mg m^{-2} IV × 2	VCR 1.3 mg m^{-2} IV × 3		
CPM 700 mg m^{-2} IV × 2	PSL 30 mg m^{-2} PO × 10		
PSL 40–60 mg m^{-2} PO × 14			
IT × 1	MIT 6 mg m^{-2} IV × 3		
	ETP 100 mg m^{-2} IV × 5		
MIT 6 mg m^{-2} IV × 3	Ara-C 100 mg m^{-2} IV × 6		
VDS 2 mg m^{-2} IV × 2	IT × 1		
CPM 700 mg m^{-2} IV × 2			
PSL 40–60 mg m^{-2} PO × 14	MTX 600 mg m^{-2} IV × 2		
IT × 1	L-ASP 10000 IU m^{-2} IV × 2		
	IT × 1		
ADR 20 mg m^{-2} IV × 3			
VDS 2 mg m^{-2} IV × 2	ACR 14 mg m^{-2} IV × 8		
CPM 700 mg m^{-2} IV × 2	Ara-C 70 mg m^{-2} IV × 7		
PSL 40–60 mg m^{-2} PO × 14	PSL 40 mg m^{-2} PO × 7		
IT × 1	IT × 1		
Maintenance			
6MP 60 mg m^{-2} PO daily	6MP 60 mg m^{-2} PO daily	VCR 1.3 mg m^{-2} IV monthly	
MTX 20 mg m^{-2} PO weekly	MTX 20 mg m^{-2} PO weekly	PSL 60 mg m^{-2} PO × 5 monthly	
		6MP 60 mg m^{-2} PO daily	
		MTX 20 mg m^{-2} PO weekly	

Maximum dose of VCR was 2.0 mg/body. For remission induction in the ALL90 study, number of doses for each drug was determined according to the findings of serial bone marrow aspirations. Drugs used for IT injection were MTX 15 mg/body, Ara-C 40 mg/body and PSL 10 mg/body in the ALL90/ALL93 studies, and MTX 15 mg/body and DEX 4 mg/body with or without Ara-C 40 mg/body in the ALL97 study. ALL, acute lymphoblastic leukemia; VCR, vincristine; ADR, doxorubicin; CPM, cyclophosphamide; PSL, prednisolone; L-ASP, L-asparaginase; MIT, mitoxantrone; DNR, daunorubicin; ETP, etoposide; BHAC, behenoyl-ara-C; VDS, vindesine; DEX, dexamethasone; Ara-C, cytarabine; ACR, aclarubicin; MTX, methotrexate; 6MP, 6-mercaptoprine; IV, intravenous; PO, oral; SC, subcutaneous; IM, intramuscular; IT, intrathecal.

between the curves were compared using a log-rank test. For risk factor analysis, a multivariate Cox proportional hazards model was constructed for OS, and a logistic regression model for CR achievement. Variables with p-values of less than 0.10 by log-rank test for OS, and in univariate logistic analysis for CR achievement were included in the respective final multivariate model. A hazard ratio (HR) and an odds ratio (OR) were calculated in conjunction with a 95% confidence interval (CI). Stata Version 8 software (StataCorp, College Station, TX, USA) was used for all statistical analyses.

3. Results

3.1. Patients

Among 559 patients whose immunophenotypes were evaluable, 87 (15.6%) were identified as T-ALL. Baseline characteristics of the 87 patients are summarized in Table 2. The median age was 26 years (range, 15–60 years), with 60 males and 27 females. Involvements in CNS, skin, and mediastinum were detected in 7.0, 4.6 and 17.2%, respectively. Of the 60 patients for which cytogenetic information was available, 34 showed abnormal karyotype, including del(5q) in 4, del(6q) in 3, del(9p) in 3, del(11q) in 3, t(11;14) in 2, t(1;12) in 2, trisomy 8 in 2, and del(12p) in 2 patients.

3.2. Response to induction therapy

In total, 66 patients (75.8%) achieved CR after one course of remission induction therapy ($n=59$), or two courses ($n=7$). Of the remaining 21 patients, toxicity-related death during induction therapy occurred in four (4.6%). Their causes of death were sepsis ($n=2$), intracranial hemorrhage ($n=1$), and liver failure ($n=1$). Multivariate analysis indicated two factors were significantly associated with CR achievement. Patients aged 30 or older had a greater risk of induction failure (OR: 5.13, 95% CI: 1.52–17.5, $p=0.009$), as did those whose serum albumin level was lower than 3.5 g dl^{-1} (OR: 6.71, 95% CI: 1.64–27.4, $p=0.008$). Other variables such as initial WBC count, sex, performance status, and any surface markers did not correlate with CR achievement.

Table 2
Presenting characteristics

	$n=87$
Age (years)	26 (15–60)
Sex: male/female	60/27
FAB type: L1/L2	27/60
WBC count ($\times 10^9\ l^{-1}$l)	17.1 (0.3–396)
RBC count ($\times 10^{12}\ l^{-1}$)	3.20 (1.49–6.33)
Platelet count ($\times 10^9\ l^{-1}$)	57 (4–341)
Performance status: 0–1/2–3	66/17
CNS involvement: present/absent	6/80
Skin involvement: present/absent	4/83
Mediastinal involvement: present/absent	15/72
Karyotype: normal/abnormal/NE	26/34/27

Values are presented as median (range) unless indicated. FAB, French–American–British; WBC, white blood cell; RBC, red blood cell; CNS, central nervous system; NE, not evaluable (not carried out or failed).

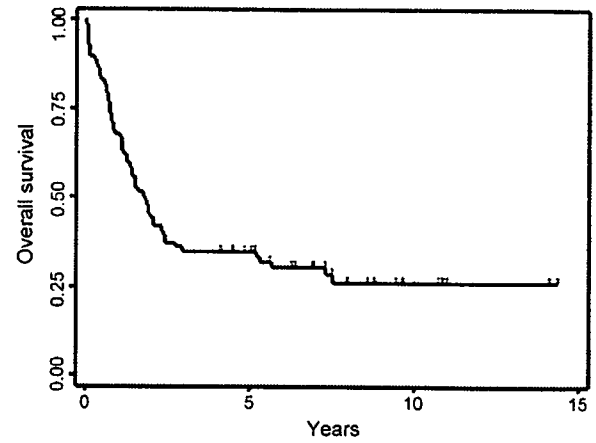

Fig. 1. Kaplan–Meier curve for overall survival. The probability of overall survival was 35.0% at 5 years for the whole population ($n=87$).

3.3. Survival

At the end of observation, 26 patients were alive with a median follow-up of 7.5 years (range, 0.8–14.3 years). Among 66 remitters, relapse occurred in 41 patients. A total of 33 patients underwent allogeneic HSCT, and three underwent autologous HSCT. Disease status at the time of transplantation was first CR for 17, second CR for 7, third CR for 1, non-CR for 9, and unknown for 2.

Fig. 1 shows the survivals for all patients. The probability of OS at 5 years was $35.0 \pm 5.1\%$ for the whole population. As there was no survival difference among the three studies ($p=0.475$), nor between the two studies (data not shown), all patients were grouped for the risk factor analysis. OS according to patient characteristics is presented in Table 3. Univariate analysis showed that their initial WBC counts and serum albumin levels strongly affected survival. As presented in Fig. 2, patients with a WBC count of $50 \times 10^9\ l^{-1}$ or higher had lower survival ($19.2 \pm 7.7\%$ at 5 years). Unexpectedly, the outcome for those with a WBC count lower than $3 \times 10^9\ l^{-1}$ was also worse ($20.0 \pm 10.0\%$ at 5 years) than those with an intermediate count ($48.0 \pm 7.5\%$ at 5 years). Induction failure or disease recurrence occurred in 14 of 15 patients in the low-count group, in 26 of 45 in the intermediate-count group, and in 22 of 27 in the high-count group. These observations resulted in inferior EFS rates for those in the low- and high-count groups to those in the intermediate-count group, too (Fig. 3). Even when patients undergoing HSCT were analyzed as censored cases at the time of transplantation, differences in terms of both OS and

Table 3
Overall survival at 5 years according to patient characteristics

Characteristics	Number of patients	Overall survival (%)	p-Value
All cases	87	35.0 ± 5.1	
Treatment protocol			0.475
ALL90	21	33.3 ± 10.3	
ALL93	26	44.7 ± 9.9	
ALL97	40	30.0 ± 7.2	
Age			0.054
Younger than 30	50	42.0 ± 7.0	
30 or older	37	25.4 ± 7.3	
Sex			0.700
Male	60	31.7 ± 6.0	
Female	27	42.9 ± 9.7	
WBC count			0.003
Lower than $3 \times 10^9\,l^{-1}$	15	20.0 ± 10.0	
3×10^9–$50 \times 10^9\,l^{-1}$	45	48.0 ± 7.5	
$50 \times 10^9\,l^{-1}$ or higher	27	19.2 ± 7.7	
Serum albumin			<0.001
Lower than 3.5 g dl^{-1}	14	7.1 ± 6.9	
3.5 g dl^{-1} or higher	68	39.0 ± 6.0	
Performance status			0.577
0–1	66	37.1 ± 6.0	
2–3	17	29.4 ± 11.1	
CNS involvement			<0.001
Present	6	0.0 ± 0.0	
Absent	80	38.1 ± 5.5	
Skin involvement			<0.001
Present	4	0.0 ± 0.0	
Absent	83	36.7 ± 5.3	
Mediastinal involvement			0.077
Present	15	53.3 ± 12.9	
Absent	72	31.2 ± 5.5	
No. of induction course[a]			0.972
1 Course	59	44.9 ± 6.5	
2 Courses	7	42.9 ± 18.7	

ALL, acute lymphoblastic leukemia; WBC, white blood cell; CNS, central nervous system. Values are presented with standard errors.
[a] Only patients who achieved complete remission are considered.

EFS remained statistically significant (data not shown). Of the 14 patients with serum albumin lower than 3.5 g dl^{-1}, seven failed to obtain CR, and all of the remaining patients with CR had a relapse. The probability of survival for these patients was only 7.1 ± 6.9% at 5 years. An age of 30 or older, and the presence of mediastinal involvement were also associated with a trend in favor of survival. Although the number was small, patients who presented CNS or skin involvement had an extremely poor prognosis, and no long-term survivors existed. We failed to detect a significant effect of sex, performance status, or number of induction courses on survival. Neither did surface markers including CD2, CD3, CD34, or myeloid antigens have any prognostic significance. Based on these results, serum albumin levels, initial WBC counts, age, and mediastinal involvement were subjected to a multivariate analysis. The results are shown in Table 4. Lower albumin levels, too low or too high WBC counts, and older age were identified as independently associated with lower survival.

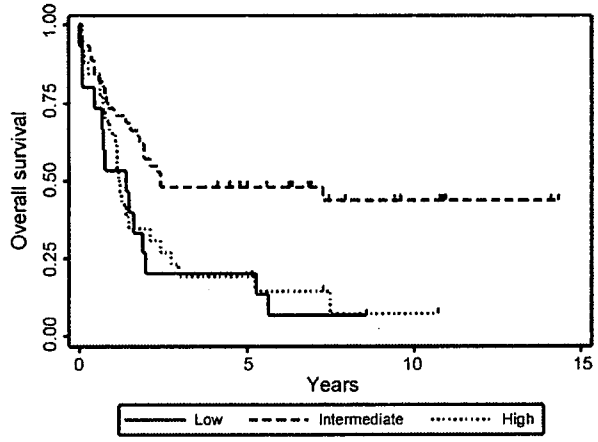

Fig. 2. Kaplan–Meier curves for overall survival according to the initial white blood cell count. The low-count group (less than $3 \times 10^9\,l^{-1}$, $n = 15$) as well as the high-count group ($50 \times 10^9\,l^{-1}$ or higher, $n = 27$) showed significantly worse overall survival than the intermediate-count group (3–$50 \times 10^9\,l^{-1}$, $n = 45$: $p = 0.0037$ and 0.0055, respectively).

3.4. Outcome after relapse

A total of 41 remitters had a disease recurrence after a median CR duration of 8.6 months (range, 0.6–79.4 months). The sites of relapse were BM in 28, CNS in 9, concurrent BM and CNS in 2, intraocular area in one, and mamma in one. Among 11 cases whose disease recurred in CNS, none had CNS involvement at presentation. The probabilities of OS for the whole recurred patients were 26.7 ± 7.1% at 1 year, and 11.9 ± 5.4% at 5 years after relapse. The survival curves are shown in Fig. 4. No patients could survive long-term unless they underwent HSCT after relapse.

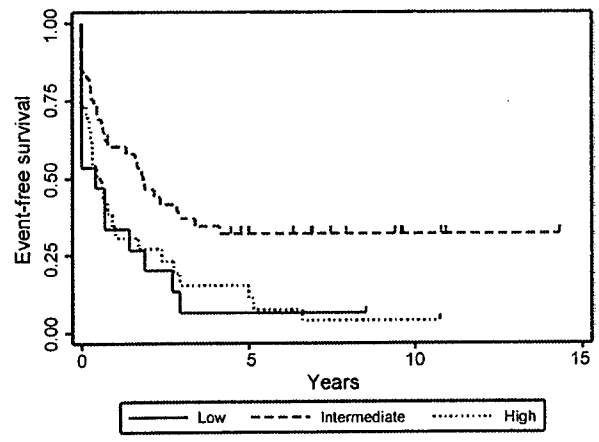

Fig. 3. Kaplan–Meier curves for event-free survival according to the initial white blood cell count. The low-count group (less than $3 \times 10^9\,l^{-1}$, $n = 15$) as well as the high-count group ($50 \times 10^9\,l^{-1}$ or higher, $n = 27$) showed significantly worse event-free survival than the intermediate-count group (3–$50 \times 10^9\,l^{-1}$, $n = 45$: $p = 0.0146$ and 0.0221, respectively).

Table 4
Factors associated with overall survival

Univariate analysis p-value	Multivariate analysis		
	p-Value	HR (95% CI)	Factor
Serum albumin			
<0.001	0.013	2.25 (1.18–4.28)	Lower than 3.5 g dl^{-1}
		1.00	3.5 g dl^{-1} or higher
WBC count			
0.004	0.018	2.34 (1.16–4.73)	50 × 10^9 l^{-1} or higher
0.005	0.036	1.91 (1.04–3.53)	Lower than 3 × 10^9 l^{-1}
		1.00	3 × 10^9–50 × 10^9 l^{-1}
Age			
0.057	0.049	1.70 (1.00–2.89)	30 or older
		1.00	Younger than 30
Mediastinal involvement			
0.084	0.212	1.68 (0.74–3.80)	Absent
		1.00	Present

An HR higher than unity indicates worse survival for patients with the factor. HR, hazard ratio; 95% CI, 95% confidence interval; WBC, white blood cell.

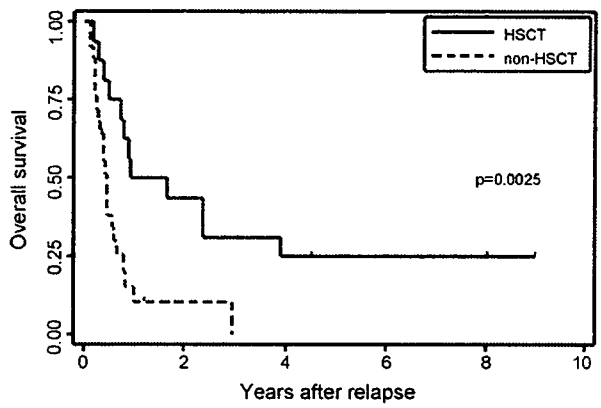

Fig. 4. Kaplan–Meier curves for survival after relapse The 5-year probability of overall survival after relapse was 25.0% for patients who underwent transplantation thereafter (n = 16), and 0% for those who did not (n = 25).

4. Discussion

A recent meta-analysis [15] showed that patients with high-risk ALL benefit from allogeneic HSCT during first CR, whereas its efficacy is unclear for those with standard-risk ALL, suggesting the importance of prognostic prediction at diagnosis or soon thereafter. Risk stratification for ALL has been traditionally studied without distinguishing T-ALL from B-lineage ALL; however, because they are two distinct clinical entities, determining prognostic factors separately should mean more accuracy. But the relatively small number of patients, especially those with T-ALL, available for a single trial has made such an analytical examination difficult. In this study, through combining data for patients from the three prospective JALSG trials, we studied long-term outcomes and prognostic factors specific for T-ALL.

Given that highly intensive regimens in recent reports have increased survival rates for adult T-ALL up to 40–60% [8–11], the 5-year survival rate of 35% for our patients was somewhat low. As it is suggested that CPM and Ara-C may play an important role in the treatment of T-ALL [8], our results would leave room for improvement through the use of treatment that features intensified administration of such agents. Risk factor analysis identified serum albumin levels, initial WBC counts, and age as having independent values for predicting survival. As reported previously, an initial WBC count had significant influence on survival, but the striking finding was that not only the high-count group (50 × 10^9 l^{-1} or higher) but also the low-count group (less than 3 × 10^9 l^{-1}) showed a significantly worse survival rate than the intermediate-count group (p = 0.0055 and 0.0037, respectively). This observation is in accordance with the report from the Pediatric Oncology Group (POG) [16]. They described that the subgroup of patients with T-ALL who had an initial WBC count of less than 10 × 10^9 l^{-1} at diagnosis fared worse than those with a WBC count between 10 × 10^9 and 50 × 10^9 l^{-1}. It is an accepted concept that a high WBC count has less influence on the prognosis of T-ALL than of B-lineage ALL [8]. Many investigators have pursued the upper cut-off points to discriminate outcomes, with these cut-off points recently set at 100 × 10^9 l^{-1} in adults [7,8], which is much higher than for B-lineage ALL. From the results of both POG and our studies, it can be assumed that a poor prognosis for T-ALL patients with a low WBC count may partly offset the prognostic significance of the high WBC count. Possible reasons why a low WBC count affected survival adversely could not be identified from a careful examination of our patient data. A French group showed that the T-cell receptor (TCR) status could stratify T-ALL into four groups, and patients in the immature subset presented a lower WBC count, and had inferior survival mainly due to a lower CR rate [17]. However, for our patients, a low WBC count did not exert any significant effect on CR achievement, but was associated with shorter survival due to a higher rate of relapse. Immunophenotypic maturation stages have also been indicated in correlating with the outcome for T-ALL [16,18–21], although

interactions between such maturation stages and initial WBC counts have not been established. Lack of information for CD1a expression in our dataset enabled us to classify our patients according to the criteria by the European Group for the Immunological Characterization of Leukemias (EGIL) [22]; however, the observations that expression of surface markers including CD3 and CD34 revealed no prognostic relevance seem to show that worse outcomes for patients with low WBC counts cannot be explained by maturation stages. More recently, risk assessment for T-ALL has been investigated based on the genetic characteristics of leukemic cells, including the expression of specific genes such as HOX11 [23,24] and HOX11L2 [25], gene expression profiles using microarray technology [26], and DNA methylation profiles [27]. Also, prognostic significance of minimal residual disease (MRD) during or after treatment has been vigorously studied, and several groups showed clinical utility of MRD quantification by flow cytometry [28,29] or polymerase chain reaction [30–32]. Although such research should be continued, risk assessment according to information commonly available at all hospitals remains important in clinical practice. It should be noted that our study has several limitations, and the results must be interpreted with caution. The limitations include the retrospective nature of the study, and the relatively small number of patients, especially of those in the low WBC count group ($n = 15$). Validations for a larger number of patients will be needed.

In summary, from the analysis of a relatively large cohort of 87 adult patients with T-ALL, serum albumin levels, initial WBC counts, and age were identified as prognostic factors for survival. For WBC counts, not only patients with a high count, but also those with a low count had significantly worse outcomes than patients with an intermediate count. Although our findings need confirmation, these results will be helpful in the identification of prognostically distinct subgroups within adult T-ALL.

Acknowledgements

We would like to thank all physicians and staff at the cooperating centers. This study was supported in part by the Grants for Cancer from Ministry of Health, Welfare and Labor and by the Grant for Cancer Translational Research Project from Ministry of Education, Culture, Sports, Science and Technology, Government of Japan.

References

[1] Hoelzer D, Thiel E, Ludwig WD, Loffler H, Buchner T, Freund M, et al. Follow-up of the first two successive German multicentre trials for adult ALL (01/81 and 02/84). German Adult ALL Study Group. Leukemia 1993;7(Suppl. 2):S130–4.

[2] Larson RA, Dodge RK, Burns CP, Lee EJ, Stone RM, Schulman P, et al. A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. Blood 1995;85:2025–37.

[3] Durrant IJ, Prentice HG, Richards SM. Intensification of treatment for adults with acute lymphoblastic leukaemia: results of U.K. Medical Research Council randomized trial UKALL XA. Medical Research Council Working Party on Leukaemia in Adults. Br J Haematol 1997;99:84–92.

[4] Kantarjian HM, O'Brien S, Smith TL, Cortes J, Giles FJ, Beran M, et al. Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. J Clin Oncol 2000;18:547–61.

[5] Annino L, Vegna ML, Camera A, Specchia G, Visani G, Fioritoni G, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. Blood 2002;99:863–71.

[6] Takeuchi J, Kyo T, Naito K, Sao H, Takahashi M, Miyawaki S, et al. Induction therapy by frequent administration of doxorubicin with four other drugs, followed by intensive consolidation and maintenance therapy for adult acute lymphoblastic leukemia: the JALSG-ALL93 study. Leukemia 2002;16:1259–66.

[7] Rowe JM, Buck G, Burnett AK, Chopra R, Wiernik PH, Richards SM, et al. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. Blood 2005;106:3760–7.

[8] Hoelzer D, Gokbuget N. Recent approaches in acute lymphoblastic leukemia in adults. Crit Rev Oncol Hematol 2000;36:49–58.

[9] Bassan R, Gatta G, Tondini C, Willemze R. Adult acute lymphoblastic leukaemia. Crit Rev Oncol Hematol 2004;50:223–61.

[10] Boucheix C, David B, Sebban C, Racadot E, Bene MC, Bernard A, et al. Immunophenotype of adult acute lymphoblastic leukemia, clinical parameters, and outcome: an analysis of a prospective trial including 562 tested patients (LALA87). French Group on therapy for adult acute lymphoblastic leukemia. Blood 1994;84:1603–12.

[11] Czuczman MS, Dodge RK, Stewart CC, Frankel SR, Davey FR, Powell BL, et al. Value of immunophenotype in intensively treated adult acute lymphoblastic leukemia: cancer and leukemia Group B study 8364. Blood 1999;93:3931–9.

[12] Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol 2005;23:6306–15.

[13] Ueda T, Miyawaki S, Asou N, Kuraishi Y, Hiraoka A, Kuriyama K, et al. Response-oriented individualized induction therapy with six drugs followed by four courses of intensive consolidation, 1 year maintenance and intensification therapy: the ALL90 study of the Japan Adult Leukemia Study Group. Int J Hematol 1998;68:279–89.

[14] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. The morphological classification of acute lymphoblastic leukaemia: concordance among observers and clinical correlations. Br J Haematol 1981;47:553–61.

[15] Yanada M, Matsuo K, Suzuki T, Naoe T. Allogeneic hematopoietic stem cell transplantation as part of postremission therapy improves survival for adult patients with high-risk acute lymphoblastic leukemia: a metaanalysis. Cancer 2006;106:2657–63.

[16] Pullen J, Shuster JJ, Link M, Borowitz M, Amylon M, Carroll AJ, et al. Significance of commonly used prognostic factors differs for children with T cell acute lymphocytic leukemia (ALL), as compared to those with B-precursor ALL. A Pediatric Oncology Group (POG) study. Leukemia 1999;13:1696–707.

[17] Asnafi V, Buzyn A, Thomas X, Huguet F, Vey N, Boiron JM, et al. Impact of TCR status and genotype on outcome in adult T-cell acute lymphoblastic leukemia: a LALA-94 study. Blood 2005;105:3072–8.

[18] Thiel E, Kranz BR, Raghavachar A, Bartram CR, Loffler H, Messerer D, et al. Prethymic phenotype and genotype of pre-T (CD7+/ER-)-cell leukemia and its clinical significance within adult acute lymphoblastic leukemia. Blood 1989;73:1247–58.

[19] Garand R, Voisin S, Papin S, Praloran V, Lenormand B, Favre M, et al. Characteristics of pro-T ALL subgroups: comparison with late T-ALL. The Groupe d'Etude Immunologique des Leucemies. Leukemia 1993;7:161–7.

[20] Uckun FM, Gaynon PS, Sensel MG, Nachman J, Trigg ME, Steinherz PG, et al. Clinical features and treatment outcome of childhood

T-lineage acute lymphoblastic leukemia according to the apparent maturational stage of T-lineage leukemic blasts: a Children's Cancer Group study. J Clin Oncol 1997;15:2214–21.
[21] Vitale A, Guarini A, Ariola C, Mancini M, Mecucci C, Cuneo A, et al. Adult T-cell acute lymphoblastic leukemia: biologic profile at presentation and correlation with response to induction treatment in patients enrolled in the GIMEMA LAL 0496 protocol. Blood 2006;107:473–9.
[22] Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the immunological characterization of leukemias (EGIL). Leukemia 1995;9:1783–6.
[23] Kees UR, Heerema NA, Kumar R, Watt PM, Baker DL, La MK, et al. Expression of HOX11 in childhood T-lineage acute lymphoblastic leukaemia can occur in the absence of cytogenetic aberration at 10q24: a study from the Children's Cancer Group (CCG). Leukemia 2003;17:887–93.
[24] Ferrando AA, Neuberg DS, Dodge RK, Paietta E, Larson RA, Wiernik PH, et al. Prognostic importance of TLX1 (HOX11) oncogene expression in adults with T-cell acute lymphoblastic leukaemia. Lancet 2004;363:535–6.
[25] Cave H, Suciu S, Preudhomme C, Poppe B, Robert A, Uyttebroeck A, et al. Clinical significance of HOX11L2 expression linked to t(5;14)(q35;q32), of HOX11 expression, and of SIL-TAL fusion in childhood T-cell malignancies: results of EORTC studies 58881 and 58951. Blood 2004;103:442–50.
[26] Chiaretti S, Li X, Gentleman R, Vitale A, Vignetti M, Mandelli F, et al. Gene expression profile of adult T-cell acute lymphocytic leukemia identifies distinct subsets of patients with different response to therapy and survival. Blood 2004;103:2771–8.
[27] Roman-Gomez J, Jimenez-Velasco A, Agirre X, Prosper F, Heiniger A, Torres A. Lack of CpG island methylator phenotype defines a clinical subtype of T-cell acute lymphoblastic leukemia associated with good prognosis. J Clin Oncol 2005;23:7043–9.
[28] Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 1998;351: 550–4.
[29] Dworzak MN, Froschl G, Printz D, Mann G, Potschger U, Muhlegger N, et al. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood 2002;99:1952–8.
[30] Cave H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukaemia. European Organization for Research and Treatment of Cancer—Childhood Leukemia Cooperative Group. N Engl J Med 1998;339:591–8.
[31] van Dongen JJ, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemse MJ, Corral L, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998;352:1731–8.
[32] Bruggemann M, Raff T, Flohr T, Gokbuget N, Nakao M, Droese J, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood 2006;107:1116–23.

International Journal of
HEMATOLOGY

Imatinib Provides Durable Molecular and Cytogenetic Responses in a Practical Setting for Both Newly Diagnosed and Previously Treated Chronic Myelogenous Leukemia: A Study in Nagasaki Prefecture, Japan

Emi Matsuo,[a] Yasushi Miyazaki,[a] Chizuko Tsutsumi,[a] Yoriko Inoue,[a] Reishi Yamasaki,[a] Tomoko Hata,[a] Takuya Fukushima,[a] Hideki Tsushima,[a] Daisuke Imanishi,[a] Yoshitaka Imaizumi,[a] Masako Iwanaga,[a] Mari Sakai,[a] Koji Ando,[a] Yasushi Sawayama,[a] Daisuke Ogawa,[a] Yasuhisa Kawaguchi,[a] Kazuhiro Nagai,[a] Kunihiro Tsukasaki,[a] Shuichi Ikeda,[b] Yukiyoshi Moriuchi,[b] Shinichiro Yoshida,[b] Miyuki Honda,[b] Jun Taguchi,[b] Yasuyuki Onimaru,[b] Takeshi Tsuchiya,[b] Masayuki Tawara,[b] Sunao Atogami,[b] Masaomi Yamamura,[b] Hisashi Soda,[b] Yoshiharu Yoshida,[b] Yuji Matsuo,[b] Hiroaki Nonaka,[b] Tatsuro Joh,[b] Yumi Takasaki,[b] Chiyuki Kawasaki,[b] Saburo Momita,[b] Itsuro Jinnai,[b] Kazutaka Kuriyama,[b] Masao Tomonaga[a]

[a]Department of Hematology and Molecular Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [b]Nagasaki CML Study Group, Nagasaki, Japan

Received August 1, 2006; received in revised form November 17, 2006; accepted December 1, 2006

Abstract

To evaluate the efficacy of imatinib in a practical setting, we registered 43 patients with newly diagnosed chronic myelogenous leukemia (CML) (group I) and 56 patients with previously diagnosed CML (group II) at 11 hematology centers in Nagasaki prefecture, Japan, from December 2001 to July 2005 and analyzed the molecular responses. Cytopenia, fluid retention, and skin rash were major adverse events, along with elevation in creatine phosphokinase levels. With a follow-up of approximately 3.5 years, imatinib treatment led to 88.7% overall survival (OS) and 85.2% progression-free survival (PFS) rates for group I, and 79.8% OS and 76.6% PFS rates for group II; the rates were not significantly different despite a lower average imatinib dose in group II. The rates of complete cytogenetic response at 30 months and major molecular response at 24 months were 86.1% and 62.5%, respectively, in group I, and 77.9% and 58.3% in group II; the rates were not significantly different. As has been reported by other groups, these results demonstrate that imatinib treatment can provide excellent clinical and molecular effects for not only newly diagnosed but also previously treated CML patients in practical settings that cover a wider variety of patients than clinical trials.

Int J Hematol. 2007;85:132-139. doi: 10.1532/IJH97.06157

Key words: Imatinib; Chronic myelogenous leukemia; Molecular response; Cytogenetic response; New diagnosis; Previous diagnosis

1. Introduction

Treatment for chronic myelogenous leukemia (CML) has changed dramatically since the introduction of imatinib [1]. Imatinib blocks the activity of the specific Philadelphia chromosome (Ph)-derived BCR-ABL fusion protein, which confers a strong growth advantage upon Ph-positive CML cells [1-3]. Reflecting in vitro observations are many reports regarding the clinical effectiveness of imatinib on Ph-positive leukemias [4-8]. For example, the International Randomised Study of Interferon versus STI571 (IRIS) study [4], a phase III clinical trial that compared imatinib treatment and treatment with interferon (IFN) plus cytosine arabinoside for CML patients in the first chronic phase, clearly demonstrated the excellent clinical and cytogenetic/molecular effects of imatinib.

See "Appendix" for affiliations of the members of the Nagasaki CML Study Group.

Correspondence and reprint requests: Yasushi Miyazaki, MD, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; 81-(0)95-849-7111; fax: 81-(0)95-849-7113 (e-mail: y-miyaza@nagasaki-u.ac.jp).

The cytogenetic response to CML treatment, ie, reduction in Ph-positive metaphases, can be usually obtained with IFN, imatinib, or allogeneic hematopoietic stem cell transplantation (allo-HSCT), and cytogenetic evaluation is used to evaluate the response to treatment [9,10]. Approximately 80% of the patients in the chronic phase who use imatinib will achieve the disappearance of Ph-positive metaphases (a complete cytogenetic response [CCR]) [5], making a molecularly analysis necessary to precisely evaluate the efficacy of imatinib, which has been used to follow minimal residual disease, mostly after allo-HSCT [11,12]. On the basis of the reports of many clinical trials, imatinib has become the first choice of treatment for patients with newly diagnosed CML [1].

Patients participating in clinical trials are usually selected according to strict eligibility criteria. These regulations are widely accepted as necessary in clinical trials to clearly answer the questions asked and to ensure the protection of the patients' safety and rights. In practical situations, however, the clinical features of patients are much more heterogeneous than those defined by the selection criteria in clinical trials [13]. For example, physicians need to treat patients who range from young, previously untreated patients to elderly patients who have had prior treatments and who may have many accompanying diseases and organ dysfunctions that sometimes may not allow use of the recommended drug doses.

At the time imatinib became widely available in Japan (December 2001), we wanted to know whether imatinib could reproduce in a practical setting the high efficacy that had been reported in clinical trials. To answer this question, we followed as many patients as possible over a wide variety of CML patients in Nagasaki prefecture, Japan, and we evaluated the efficacy of imatinib with molecular techniques. We also investigated how the treatment for CML changed with imatinib therapy in the same area.

By following almost 100 patients for more than 3.5 years, this study demonstrated the significantly higher efficacy and excellent clinical effects of imatinib treatment in a practical setting for both patients with newly diagnosed CML and those with previously treated CML.

2. Patients and Methods

2.1. Patients

The 99 CML patients who participated in this study were reported from 11 hospitals in Nagasaki prefecture and included (1) patients with CML newly diagnosed between December 2001 and July 2005 and (2) patients alive at the beginning of this study (December 2001). Informed consent was obtained from 78 of the 99 patients to measure the amount of bcr-abl fusion transcripts and to analyze gene mutation of the abl kinase domain in peripheral blood or bone marrow samples. In all, 554 samples were collected (250 peripheral blood and 304 bone marrow samples).

2.2. RNA Extraction, Complementary DNA Synthesis, and Polymerase Chain Reaction Conditions

Peripheral blood and bone marrow samples were sent to the Department of Hematology, Nagasaki University, where all measurements of bcr-abl fusion transcript amounts and sequence analyses of the kinase domain of the abl gene were performed. Mononuclear cells were separated from samples and disrupted to extract total RNA with the RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized from total RNA with random hexamer primers and a ProSTAR First Strand RT-PCR kit (Stratagene, La Jolla, CA, USA) for quantification of the bcr-abl fusion gene.

Quantitative real-time reverse transcriptase–polymerase chain reaction (RQ-PCR) analysis was performed with the LightCycler (Roche Diagnostics, Mannheim, Germany) and LightCycler Fast Start DNA Master SYBR Green 1 (Roche Diagnostics). PCR conditions and primer sequences are available on request. Each PCR reaction was independently performed at least twice, with monitoring of melting curves and gel electrophoresis of the products to ensure correct amplification. The amount of the fusion gene in the original sample was calculated by means of a standard curve (created with the bcr-abl fusion gene or the abl gene cloned in plasmids) and expressed as the bcr-abl/abl ratio. The lower limit of quantification was 1×10^{-4}. In several samples, the entire region of the abl kinase domain was sequenced in both forward and reverse directions by means of a BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.3. Clinical Parameters, Including Response to Therapy

Progression-free survival (PFS) was calculated from the first day of imatinib administration to the date of death, the date of development of the accelerated phase or blastic crisis of CML, or the date of the last follow-up. Overall survival (OS) was calculated from the day of diagnosis (for newly diagnosed patients, group I; see "Results") or the first day of imatinib administration (for patients with previously diagnosed CML, group II) to the date of any cause of death or the date of the last follow-up. The daily dose of imatinib was calculated as an average dose, the total amount of imatinib administered divided by the number of days of the administration period.

A CCR was defined as the absence of Ph-positive metaphases in the sample. In the event that no cytogenetic data were available but RQ-PCR or fluorescence in situ hybridization (FISH) results for bcr-abl fusion were available, we included CCR-equivalent responses (a bcr-abl/abl ratio <0.01 by RQ-PCR or below the limit of detection of bcr-abl signal by FISH) in determining the CCR rate. In terms of the molecular response, which was analyzed by RQ-PCR analysis, a 3-logarithm reduction in the data compared with those at diagnosis was categorized as a major molecular response (MMR), and the disappearance of the fusion product was defined as a complete molecular response.

2.4. Statistical Analysis

We evaluated the distribution of clinical characteristics for the 2 groups with the chi-square test or the Fisher exact test for categorical parameters and used the 2-sample

Table 1.
Clinical Characteristics of Patients in Groups I and II*

	Group I (n = 43)	Group II (n = 56)	*P*
M/F sex, n	27/16	30/26	.36
Median age at diagnosis (range), y	53 (21-71)	54 (15-74)	.56
CML phase at diagnosis, n			.36
CP	37	42	
AP	6	13	
BC	0	0	
Unknown	0	1	
Sokal score at diagnosis, n			.12
Low	19	15	
Intermediate	16	21	
High	7	13	
Unknown	1	7	
Median time after diagnosis (range), y	2.0 (0.2-3.6)	6.8 (3-22.3)	<.0001

*CML indicates chronic myelogenous leukemia; CP, chronic phase; AP, accelerated phase; BC, blastic crisis.

Student *t* test or the Wilcoxon rank sum test for continuous parameters. Imatinib doses for the 2 groups were compared with the 2-sample *t* test. The probabilities of CCR, PFS, and OS were estimated by the Kaplan-Meier methods. Comparisons of curves were performed with the log-rank test. All statistical analyses were performed with the JMP software package (SAS Institute, Cary, NC, USA). *P* values <.05 were regarded as statistically significant. All analyses were performed for data collected as of the end of July 2005.

3. Results

3.1. Number and Characteristics of Patients

During this study period (44 months, after the introduction of imatinib into clinical practice), there were 43 patients with newly diagnosed CML (group I). At the time this study started, 56 patients who had already received a CML diagnosis were alive (group II). There were no differences between the 2 groups with respect to the clinical phase of CML, Sokal score, or age at diagnosis (Table 1).

3.2. Treatments

For most patients in group I (40 of 43 patients), the initial treatment was imatinib (Figure 1). Two patients initially treated with imatinib were later changed to IFN therapy because of intolerance to imatinib. Ultimately, 42 of 43 patients in this group received imatinib at some point.

Patients in group II had received a variety of treatments until imatinib became available (Figure 1). Forty-seven patients had undergone treatment with an IFN-containing

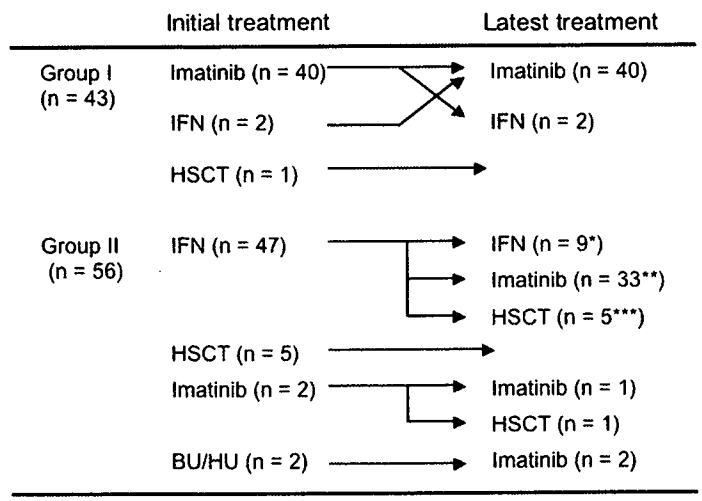

Figure 1. Initial and last treatments for patients in groups I and II. Only 1 of 43 patients in group I underwent allogeneic hematopoietic stem cell transplantation (HSCT), compared with 11 of 56 in group II (*, 1 patient discontinued interferon (IFN) treatment later; **, 2 patients discontinued imatinib treatment later; ***, 1 patient received imatinib before HSCT). Most patients were under imatinib treatment at the end of this study. BU indicates busulfan; HU, Hydrea (hydroxyurea).

Table 2.
Hematologic and Nonhematologic Toxicities of Imatinib

Hematologic toxicity (grades 3 and 4)	58.6%*
Anemia	20%
Neutropenia	34%
Thrombocytopenia	24%
Nonhematologic toxicity (grades 2 to 4)	66%*
Fluid retention	24%
Skin rash	16%
Elevation of CPK	15%
Infection	8%
Elevation of liver enzymes	5%
Fever	5%
Nausea	5%
Phlegmon	4%
Others	25%

*Several patients experienced 2 or more adverse events.

regimen at least once, and 11 patients (including 5 who had a history of IFN treatment and 1 patient who had received imatinib treatment) had undergone allo-HSCT. After imatinib became available, only 9 patients remained on IFN treatment (1 patient later discontinued IFN because of associated toxicities). Imatinib was administrated to 38 patients in group II. Four patients in this group participated in the imatinib clinical trial so that they could use the drug before it became available. One patient who received imatinib after allo-HSCT for the treatment of CML relapse achieved an MMR. Eighty of the 99 patients, including the patient treated with imatinib after HSCT, took imatinib at least once during the study period.

3.3. Toxicity of Imatinib and Complications

Hematologic toxicity of grades 3 to 4 was observed for imatinib in 41 (58.6%) of 70 evaluable patients (Table 2); most recovered after the discontinuation of imatinib treatment. Nonhematologic toxicity of grades 2 to 4 observed in 53 patients (66.3%, Table 2) necessitated treatments in several of the patients. Fluid retention (24%), skin rash (16%), and the elevation of creatine phosphokinase (CPK) levels (15%) were frequent adverse events. Of note was 1 patient who had rhabdomyolysis during imatinib treatment that resulted in death of the patient. Malignancy other than CML was diagnosed in 15 of 99 patients before (6 patients), after (5 patients), or at the same time (4 patients) CML was noticed (Figure 2). In 4 patients, gastric cancer was found during the general medical checkup performed when CML was diagnosed.

3.4. Imatinib Dosage and Molecular and Cytogenetic Responses

The time course of the molecular response to imatinib treatment is shown in Figure 4. For group I, 16 (61.5%) of 26 patients achieved an MMR by 24 months, and 20 of 32 patients (62.5%) ultimately maintained an MMR (Figure 4A). In group II, 14 (58.3%) of 24 patients achieved an MMR with imatinib treatment (Figure 4B). The 2 groups did not differ in the time required to reach a CCR (Figure 3). Among the evaluable patients, 86.1% in group I (n = 37) and 77.9% in group II (n = 31) had reached a CCR at 30 months. Table 3 summarizes the CCR and MMR data for the 2 groups.

The imatinib dosage initially was 400 mg/day in almost all patients and was later modified for a variety of reasons (Table 4). Cytopenia was the most frequently observed reason for dosage reduction, especially in group II. The imatinib dosage was reduced without toxicity of grade 3 or 4 in 10 patients, including 4 patients with no apparent adverse events. Only 16 (21.1%) of 76 patients could take 400 mg/day or more of imatinib without requiring a dosage reduction. These changes produced a difference between groups I and II in the imatinib dosage: the daily dose was significantly higher in group I (as an initial therapy) than in group II (as a second-line therapy) during the first 12 months of imatinib treatment (Table 5).

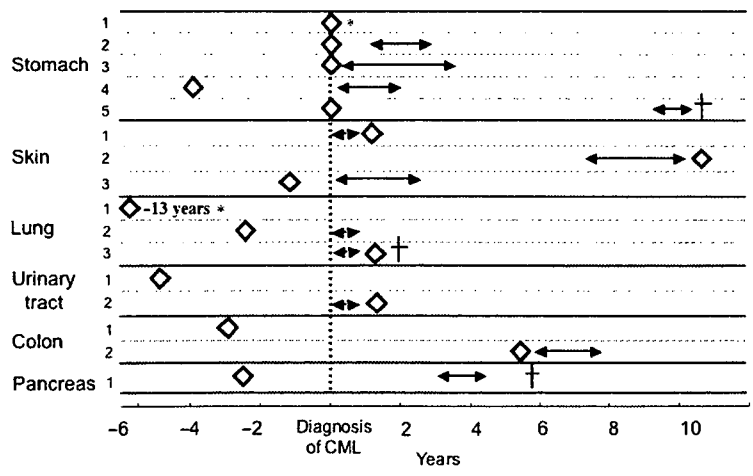

Figure 2. Malignancies other than chronic myelogenous leukemia (CML) among the patients in this study. Sixteen malignancies were found in 15 of 99 patients before, after, or at the time of CML diagnosis. Indicated are times of malignancy diagnosis (◊), the same patient (*), periods of imatinib treatment (↔), and patient death (†).

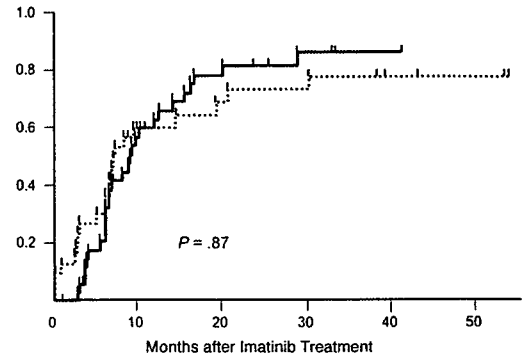

Figure 3. Accumulation of complete cytogenetic responses (CCR) in groups I and II. Time to reach CCR (and CCR equivalent as measured by real-time quantitative reverse transcriptase–polymerase chain reaction analysis) is shown. The difference between group I (n = 37, solid line) and group II (n = 31, dotted line) in the time required to obtain CCR after the start of imatinib treatment was not statistically significant.

The daily imatinib dose was related to the cytogenetic response (Figure 5). Forty-one patients (54% of patients treated with imatinib) received more than 300 mg/day of imatinib. The CCR rate at 30 months was 91.9% for 40 of these 41 patients (for whom cytogenetic data were available), 86.1% for those who received 250 to 300 mg/day of imatinib (n = 12), and 50.6% for those who received less than 250 mg/day (n = 15). There was a significant difference in CCR rate among the 3 groups (P = .0052, Figure 5). The MMR rates for the patients who received more than 300 mg/day, 250 to 300 mg/day, and less than 250 mg/day were also different in both group I and group II: 68.2%, 71.4%, and 0%, respectively, in group I and 83.3%, 75.0%, and 37% in group II. Among the 15 patients whose average daily dose was less than 250 mg, cytopenia was also the major reason for the insufficient imatinib treatment (13 of 15 patients); a CCR was achieved in only 5 of these patients. Compared with these 5 patients who achieved a CCR, patients in this category without a CCR received far less imatinib (125 mg/day versus 173 mg/day), and 4 of the 10 patients who lacked a CCR died of CML progression.

3.5. Disease Progression and Survival of Imatinib-Treated Patients

In group I, disease progression was observed in 3 patients: transition from the accelerated phase to blastic crisis in 2 patients and the acquisition of an additional cytogenetic abnormality in 1 chronic-phase patient. Two patients in group I died during imatinib treatment (from rhabdomyolysis in 1 patient, as mentioned above, and from disease complicated by lung cancer in another). In group II, disease status progressed in 8 of 33 patients treated with imatinib. Forty patients in group II were alive at the end of this study.

The OS rates at 3.5 years for imatinib-treated patients were 88.7% in group I (n = 40) and 79.8% in group II (n = 36) and were not significantly different (P = .45, Figure 6A). There was no significant difference in PFS at 3.5 years for the same patient population (85.2% in group I and 76.6% in group II; P = .51, Figure 6B).

3.6. Mutation in the Kinase Domain of the bcr-abl Fusion Gene

Of the 49 patients who lacked a complete molecular response with imatinib treatment, 10 patients did not reach a CCR. Four of these 10 patients had a point mutation in the abl kinase domain (F311I, 1 patient; T315I, 2 patients; E459K, 1 patient); these data have already been reported [14,15]. At the time we noted the mutations, 3 patients were in the accelerated phase, and 1 patient was hematologically stable but with an additional cytogenetic change. Two of these 4 patients died when their disease progressed to blastic crisis.

4. Discussion

To follow CML patients in this study with no or minimal selection bias, we had to register as many CML patients as possible throughout an entire prefecture (Nagasaki prefecture, population approximately 1.5 million). This strategy allowed us to evaluate the clinical usefulness of imatinib in the context of a daily clinical setting. In 2002, 19 CML

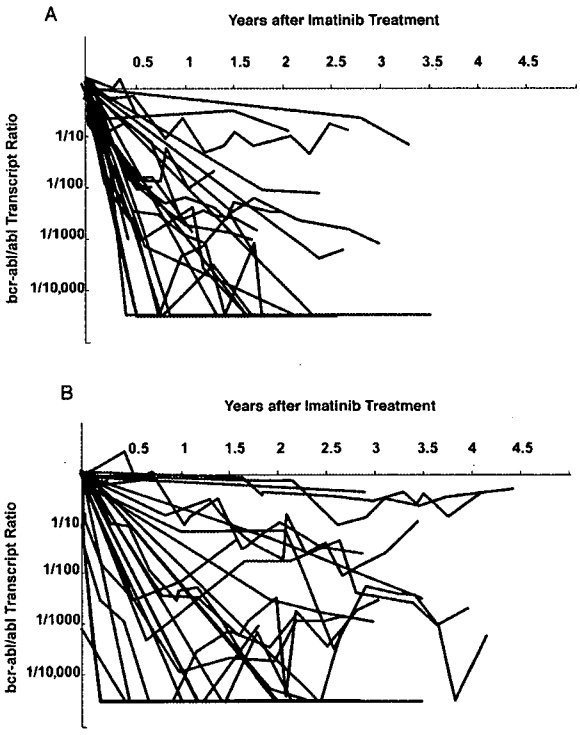

Figure 4. Reductions in bcr-abl fusion transcripts among imatinib-treated patients in group I (A) and group II (B). The changes in the amount of bcr-abl transcripts as measured by real-time quantitative reverse transcriptase–polymerase chain reaction analysis and the times after imatinib treatment are shown. Each line represents 1 patient.

Table 3.
Complete Cytogenetic Response (CCR) and Major Molecular Response (MMR) in Groups I and II

	Group I		Group II	
Time after imatinib treatment, mo	CCR, %	MMR, %	CCR, %	MMR, %
6	32.2	10.3	36.4	16.7
12	63.0	34.7	60.1	33.6
24	81.5	63.1	73.4	51.3

patients were reported to the Nagasaki Prefecture Cancer Registry (NPCR), whereas 11 patients were registered in this study. Patients who received their diagnoses outside of Nagasaki prefecture but who lived in Nagasaki prefecture and patients who received their diagnoses and were followed at home were very difficult to include. From 1985 to 1998, 187 patients with newly diagnosed CML were recorded in the NPCR (13.4 patients/year) [16], and the number of patients with newly diagnosed CML was 11.7/year in this study (43 patients during 44 months). These data indicate that our study did not include all CML patients but did cover a sufficient variety of CML patients to evaluate the practical usefulness of imatinib. Compared with the IRIS study, more patients in this study were older than 60 years (37.4% versus 21.9% in the IRIS study), and fewer patients had a low Sokal score (37.4% in this study and 50.4% in the IRIS study).

This study clearly reproduced the imatinib efficacy results described in the IRIS study, not only for patients with newly diagnosed CML but also for those with prior therapy who might include more patients in the late chronic phase. The rates of OS (88.7% at 3.5 years, n = 43) and CCR (86.1% at 30 months) for imatinib-treated patients in group I were comparable with those reported in the IRIS study (OS, 97.2% at 18 months) [4,5] and in subsequent reports [1,2,17,18].

The profiles of adverse events in this study were also similar to those in the IRIS study, and most patients could tolerate these events without the discontinuation of imatinib treatment. However, we did observe the elevation of CPK levels (grades 1 to 4) in 42% of the patients. Although not clearly mentioned in previous reports, monitoring CPK levels and muscular symptoms during imatinib treatment seemed necessary, given that one of the patients experienced rhabdomyolysis. We observed complications of additional malignancies in 15 patients (15%) in this study, including 4 patients with gastric cancer identified at the time of CML diagnosis. Although no report has described an increase in secondary malignancies with imatinib treatment, we need to pay attention to secondary and even tertiary malignancies, because imatinib treatment will prolong the survival of CML patients and we still do not know the long-term effects of imatinib on neoplasm development.

The administered imatinib dose seemed lower than that reported in the IRIS study (median dose, 400 mg/day); in our study, only 21.1% of patients could take 400 mg/day of imatinib without a reduction in dosage. In 10 patients (13.1%), the physicians reduced the imatinib dosage without a toxicity of grade 3 or 4, probably reflecting complicated situations in practice. Cytopenia was the major reason for dosage reduction and was more frequently observed in group II (Table 4), making the average imatinib dose lower in group II (259 mg/day) than in group I (334 mg/day, Table 5). This reduction might have been caused by the slow recovery of non-Ph hematopoiesis in group II after imatinib suppression of Ph-positive cells, because group II patients had a longer history of CML than group I. Fortunately, however, we observed no difference between these 2 groups in OS and PFS, which were achieved with a lower dose than recommended. This result could be explained, at least in part, by the increment of imatinib dose with time in group II. The dose gradually increased from 234 mg/day for the first 3 months to 302 mg/day after 2 years, making the average dose more than

Table 4.
Events Related to Imatinib Dosage Reduction

Events	Group I, n	Group II, n	Total, n
Patients with adverse events of grade 3 or 4			
Cytopenia alone	8	8	16
Cytopenia plus skin rash/fluid retention	1	13	14
Cytopenia plus other events	4	4	8
Skin rash alone	1	0	1
Skin rash plus events other than cytopenia	4	3	7
Patients without adverse events of grade 3 or 4			
With minor adverse events	5	1	6
Without apparent adverse events	3	1	4
Others	1	2	3
Unknown	0	1	1
Total	27	33	60

Table 5.

Comparison of Imatinib Dosages for Groups I and II

Time after Imatinib	Group	No. of Patients	Imatinib Dosage, mg/d			P
			Average*	Range	SD	
0-3 mo	I	40	328	142-400	80.8	<.0001
	II	36	234	48-400	100.5	
4-6 mo	I	34	344	122-648	100.4	.0001
	II	33	216	0-400	149.5	
7-9 mo	I	32	324	0-400	113.7	.024
	II	33	252	0-425	137.7	
10-12 mo	I	29	336	100-600	110.2	.026
	II	31	262	0-400	141.6	
1-2 y	I	27	338	89-600	111.9	.17
	II	28	297	93-400	103.1	
>2 y	I	16	310	50-400	119.3	.85
	II	23	302	106-582	121.1	

*Average imatinib dose administered to the patients in each group during the respective periods.

250 mg/day (259 mg/day). We also need to keep in mind that group II patients were selected for having maintained their CML status in a relatively stable clinical course for a certain period (3-22.3 years) before this study began. This fact would also be a reason for the fair response to imatinib in group II, even with an average dose lower than that in group I. Patients who did not (or could not) receive 250 imatinib mg/day showed insufficient clinical results. In our experience, when cytopenia makes it difficult to maintain an average imatinib dose of more than 250 mg/day, other treatment options, including allo-HSCT, should be considered. We do not recommend reducing the imatinib dosage (to less than 400 mg/day) on the basis of these observations because many groups have reported the importance of the imatinib dosage for obtaining clinical effects [19-22]. Our data rather have demonstrated that imatinib provided benefit, even for those who could not receive a sufficient dose for a period of at least 3.5 years. From our observations, the daily dose could be

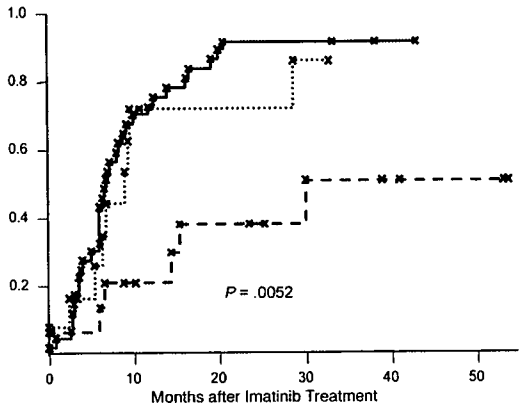

Figure 5. Time to reach a complete cytogenetic response (CCR) according to the daily imatinib dose. The differences in the times to reach a CCR (including CCR equivalent as measured by real-time quantitative reverse transcriptase–polymerase chain reaction analysis) among the 3 dosage groups were statistically significant (P = .0052). Solid line, ≥300 mg/day of imatinib (n = 40); dotted line, 250-300 mg/day (n = 12); broken line, <250 mg/day (n = 15).

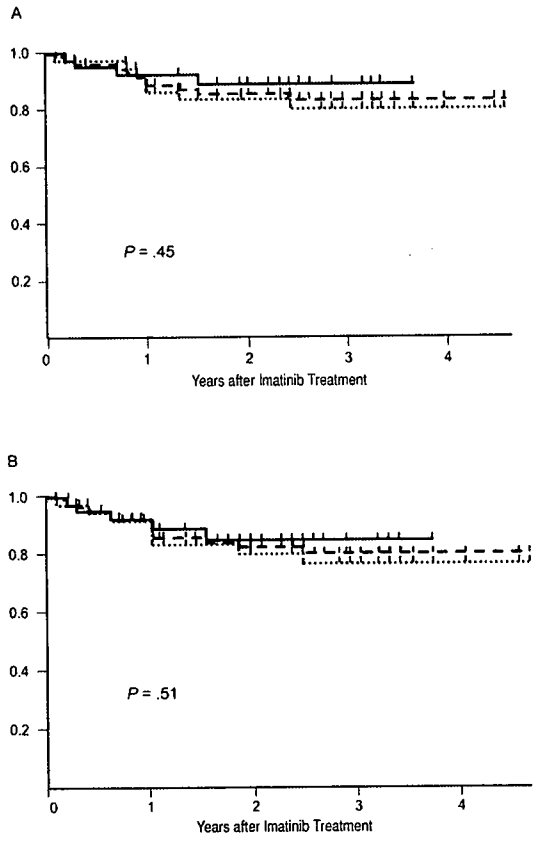

Figure 6. Overall survival (OS) (A) and progression-free survival (PFS) (B) for imatinib-treated patients in groups I and II. There were no significant differences between the 2 groups in OS and PFS. Solid line, group I; dotted line, group II; broken line, all cases.

gradually increased along with treatment for patients who could take 250 to 300 mg/day of imatinib. Imatinib could be continued at the same or an increased dosage as long as sufficient efficacy was maintained.

In summary, we reconfirmed the efficacy of imatinib in patients with CML, even in the practical setting. Imatinib will prolong the survival of CML patients in situations broader than those defined in clinical trials.

Appendix

The members of the Nagasaki CML Study Group are as follows: S. Atogami (Nagasaki Municipal Medical Center); M. Yamamura (Nagasaki Municipal Hospital); S. Momita, T. Joh, Y. Takasaki (The Japanese Red Cross Nagasaki Atomic Bomb Hospital); Y. Yoshida (St. Francis Hospital); Y. Moriuchi, J. Taguchi, T. Tsuchiya, Y. Onimaru (Sasebo Municipal General Hospital); S. Yoshida, M. Honda, M. Tawara (National Hospital Organization, Nagasaki Medical Center); Y. Matsuo (Nagasaki Prefectural Shimabara Hospital); H. Soda (Health Insurance, Isahaya General Hospital); H. Nonaka (Japan Labour Health and Welfare Organization, Nagasaki Rosai Hospital); S. Ikeda (Hirado Municipal Hospital); C. Kawasaki (Sasebo Kyosai Hospital); I. Jinnai (Saitama Medical School); K. Kuriyama (University of the Ryukyus); M. Kusano (Senju Hospital); Y. Moriwaki (Gotoh Central Hospital); and other authors at Nagasaki University.

Acknowledgment

We thank Ms. Y. Matsuo for her technical assistance.

References

1. Goldman JM, Melo JV. Chronic myeloid leukemia: advances in biology and new approaches to treatment. *N Engl J Med.* 2003;349:1451-1464.
2. Borthakur G, Cortes JE. Imatinib mesylate in the treatment of chronic myelogenous leukemia. *Int J Hematol.* 2004;79:411-419.
3. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood.* 2005;105:2640-2653.
4. O'Brien SG, Guilhot F, Larson RA, et al, for the IRIS Investigators. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2003;348:994-1004.
5. Hughes TM, Kaeda J, Branford S, et al, for the International Randomised Study of Interferon versus STI571 (IRIS) Study Group. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2003;349:1423-1432.
6. Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed *BCR-ABL*-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol.* 2006;24:460-466.
7. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood.* 2004;103:4396-4407.
8. Champagne MA, Capdeville R, Krailo M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. *Blood.* 2004;104:2655-2660.
9. Bonifazi F, de Vivo A, Rosti G, et al, for the European Study Group on Interferon in Chronic Myeloid Leukemia. Chronic myeloid leukemia and interferon-α: a study of complete cytogenetic responders. *Blood.* 2001;98:3074-3081.
10. Kim YJ, Kim DW, Lee S, et al. Comprehensive comparison of FISH, RT-PCR, and RQ-PCR for monitoring the BCR-ABL gene after hematopoietic stem cell transplantation in CML. *Eur J Haematol.* 2002;68:272-280.
11. Olavarria E, Kanfer E, Szydlo R, et al. Early detection of *BCR-ABL* transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood.* 2001;97:1560-1565.
12. Branford S, Rudzki Z, Harper A, et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. *Leukemia.* 2003;17:2401-2409.
13. Wright JR, Bouma S, Dayes I, et al. The importance of reporting patient recruitment details in phase III trials. *J Clin Oncol.* 2006;24:843-845.
14. Branford S, Rudzki Z, Walsh S, et al. Detection of *BCR-ABL* mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood.* 2003;102:276-283.
15. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, et al. Several types of mutations of the *Abl* gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood.* 2002;100:1014-1018.
16. Soda M, Ikeda T, Matsuo T, Suyama A. Cancer incidence in Nagasaki prefecture 1993-1997. In: Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, eds. *Cancer Incidence in Five Continents Vol. VIII.* Lyon, France: International Agency for Research on Cancer/International Association of Cancer Registry; 2003:390-393.
17. Cortes J, Talpaz M, O'Brien S, et al. Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with imatinib mesylate. *Clin Cancer Res.* 2005;11:3425-3432.
18. Morishima Y, Ogura M, Nishimura M, et al. Efficacy and safety of imatinib mesylate for patients in the first chronic phase of chronic myeloid leukemia: results of a Japanese phase II clinical study. *Int J Hematol.* 2004;80:261-266.
19. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med.* 2001;344:1031-1037.
20. Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood.* 2002;99:1928-1937.
21. Kantarjian HM, Talpaz M, O'Brien S, et al. Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. *Blood.* 2003;101:473-475.
22. Kantarjian H, Talpaz M, O'Brien S, et al. High-dose imatinib mesylate therapy in newly diagnosed Philadelphia chromosome-positive chronic phase chronic myeloid leukemia. *Blood.* 2004;103:2873-2878.

European Journal of Haematology ISSN 0902-4441

ORIGINAL ARTICLE

Severe hemorrhagic complications during remission induction therapy for acute promyelocytic leukemia: incidence, risk factors, and influence on outcome

Masamitsu Yanada[1], Tadashi Matsushita[1], Norio Asou[2], Yuji Kishimoto[3], Motohiro Tsuzuki[4], Yasuhiro Maeda[5], Kentaro Horikawa[2], Masaya Okada[6], Shigeki Ohtake[7], Fumiharu Yagasaki[8], Tadashi Matsumoto[9], Yukihiko Kimura[10], Katsuji Shinagawa[11], Masako Iwanaga[12], Yasushi Miyazaki[12], Ryuzo Ohno[13], Tomoki Naoe[1]

[1]Nagoya University Graduate School of Medicine, Nagoya; [2]Kumamoto University School of Medicine, Kumamoto; [3]Kansai Medical University, Moriguchi; [4]Fujita Health University School of Medicine, Toyoake; [5]Kinki University School of Medicine, Osaka-Sayama; [6]Hyogo College of Medicine, Nishinomiya; [7]Kanazawa University Hospital, Kanazawa; [8]Saitama Medical University, Saitama; [9]Jiaikai Imamura Hospital, Kagoshima; [10]Tokyo Medical University, Tokyo; [11]Okayama University Graduate School of Medicine, Okayama; [12]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; [13]Aichi Cancer Center, Nagoya, Japan

Abstract

Background: Even after the introduction of all-*trans* retinoic acid (ATRA), early hemorrhagic death remains a major cause of remission induction failure for acute promyelocytic leukemia (APL). *Methods:* To investigate severe hemorrhagic complications during remission induction therapy with respect to incidence, risk factors, and influence on outcome. Results were analyzed for 279 patients enrolled in the APL97 study conducted by the Japan Adult Leukemia Study Group (JALSG). *Results:* Severe hemorrhage occurred in 18 patients (6.5%). Although most of them were receiving frequent transfusions, the targeted levels of platelet counts (30×10^9/L) and plasma fibrinogen (1.5 g/L) for this study were reached at the day of bleeding in only 71% and 40%, respectively. Nine of them succumbed to an early death, while the remaining nine patients eventually achieved complete remission (CR). The 5-yr event-free survival rate was 68.1% for those who did not suffer severe hemorrhage, and 31.1% for those who did ($P < 0.0001$). For patients who achieved CR, on the other hand, there was no difference in disease-free survival between patients with and without severe hemorrhage ($P = 0.6043$). Risk factor analysis identified three pretreatment variables associated with severe hemorrhage: initial fibrinogen level, white blood cell count, and performance status. Additionally, patients with severe hemorrhage were more easily prone to develop retinoic acid syndrome or pneumonia than patients without hemorrhage. *Conclusions:* These results indicate that fatal hemorrhage represents a major obstacle in curing APL, and that patients with such high-risk features may benefit from more aggressive supportive care.

Key words acute promyelocytic leukemia; coagulopathy; hemorrhage; early hemorrhagic death; all-*trans* retinoic acid

Correspondence Masamitsu Yanada, MD, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa-ku, Nagoya, Aichi 466-8550, Japan. Tel.: +81 52 744 2955, Fax: +81 52 744 2801; e-mail: myanada@med.nagoya-u.ac.jp

Accepted for publication 24 November 2006

doi:10.1111/j.1600-0609.2006.00803.x

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML), characterized by specific biologic and clinical features including the presence of the t(15,17) chromosomal translocation, frequent observation of significant coagulopathy at presentation, and high susceptibility to all-*trans* retinoic acid (ATRA) (1–3). The introduction of ATRA has dramatically improved the outcome for APL, and by using current

induction therapy, complete remission (CR) rates have been increased up to 90% or higher. As a result, early hemorrhagic death is now the primary cause of remission induction failure. The frequency of fatal hemorrhage reportedly ranges from 2.4% to 11.6% (4–13), which appears to be lower than those in the pre-ATRA era. Nevertheless, severe hemorrhage, particularly in central nervous system (CNS) and lung, represents a major obstacle in curing APL. In this study, we investigated incidence, risk factors, and influence on outcome of severe hemorrhagic complications during remission induction therapy for APL by analyzing data of the patients entered into the prospective trial (APL97) conducted by the Japan Adult Leukemia Study Group (JALSG).

Patients and methods

Patients

The JALSG APL97 study enrolled patients aged between 15 and 70 yr with newly diagnosed APL. Eligibility criteria included adequate functioning of the liver [serum bilirubin level < 34.2 μM (2.0 mg/dL)], kidneys [serum creatinine level < 152.50 μM (2.0 mg/dL)], lungs ($PaO_2 \geq 60$ mmHg), and heart (no severe abnormalities detected on electrocardiograms) and an Eastern Cooperative Oncology Group performance status between 0 and 3. The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to registration.

Study design and treatments

For remission induction therapy, ATRA was administered to all patients at a daily dose of 45 mg/m^2 until CR or for 60 d, whichever was shorter. The chemotherapy protocol depended on the initial white blood cell (WBC) count and blast cell count in peripheral blood (PB). If the initial WBC count did not exceed 3.0×10^9/L and the PB blast count was less than 1.0×10^9/L (Group A), simultaneous chemotherapy was withheld. If the initial WBC count was between 3.0×10^9/L and 10.0×10^9/L and/or the PB blast count exceeded 1.0×10^9/L (Group B), patients received 12 mg/m^2 of idarubicin (IDR) on days 1 and 2, and 80 mg/m^2 of cytarabine (Ara-C) on days 1 to 5. If the initial WBC count was 10.0×10^9/L or higher (Group C), 12 mg/m^2 of IDR was administered on days 1 to 3, and 100 mg/m^2 of Ara-C on days 1 to 5. Patients whose PB blast counts exceeded 1.0×10^9/L during the induction course were given an additional 12 mg/m^2 of IDR for 2 d, and 80 mg/m^2 of Ara-C for 5 d (Group D). If patients in Group A, B, and C were treated with additional chemotherapy because of an increase in the PB blast count, they were designated as Groups AD, BD, and CD, respectively.

For the treatment of coagulopathy, platelet transfusions were administered to maintain the platelet count above 30×10^9/L, and fresh frozen plasma was transfused to maintain the plasma fibrinogen level above 1.5 g/L. Anticoagulants were used according to the discretion of the participating institutions. Retinoic acid (RA) syndrome was treated with methylprednisolone at 20 mg/kg/d for 3 d while ATRA was immediately discontinued.

Consolidation therapy consisted of three courses of intensive chemotherapy: using mitoxantrone and standard-dose Ara-C for the first course, daunorubicin, etoposide and standard-dose Ara-C for the second course, and IDR and standard-dose Ara-C for the third course. Methotrexate, Ara-C, and prednisolone were administered by intrathecal injection before the third course.

Patients who were positive for the promyelocytic-retinoic acid receptor-alpha (PML-RARα) fusion transcript in bone marrow (BM) after the completion of the consolidation therapy were treated with ATRA for 28 d followed by six courses of intensification therapy. If they were 50 yr old or younger and a suitable donor was available, allogeneic hematopoietic stem cell transplantation (HSCT) was recommended. Patients who were negative for PML-RARα after the consolidation courses were randomly assigned to either six courses of intensification therapy or no further therapy.

Evaluation of patients

CR was defined as the presence of all of the following: less than 5% of blasts in BM, no leukemic blasts in PB, recovery of PB values to neutrophil counts of at least 1.5×10^9/L and platelet counts of at least 100×10^9/L, and no evidence of extramedullary leukemia. Relapse was defined as the presence of at least one of the following: recurrence of more than 10% leukemic cells in BM or any leukemic cells in PB or extramedullary sites. Toxicity evaluation was based on the National Cancer Institute Common Toxicity Criteria Version 2.0. Severe hemorrhagic complication was defined as intracranial or pulmonary hemorrhage of grade 3 or higher. Early hemorrhagic death was defined as death during the remission induction course because of severe hemorrhagic complications.

Statistical analysis

Comparisons of baseline characteristics between patients with and without severe hemorrhage were made with the

Fisher's exact test for categorical variables, and with the Wilcoxon rank-sum test for continuous variables. Kaplan-Meier analysis was used to estimate the probability of event-free survival (EFS) and disease-free survival (DFS). EFS was defined as the time from the first day of therapy to relapse, death, or last visit, and patients who failed to achieve CR were categorized as failure cases at time zero. DFS was defined as the time from the day of achievement of CR to relapse, death, or last visit. Patients undergoing HSCT were censored at the time of transplantation. Differences between curves were compared by using a log-rank test. Cumulative incidence of severe hemorrhage was calculated with death because of other causes considered as a competing risk. To determine risk factors for the development of severe hemorrhage, variables with *P*-values of less than 0.10 in univariate logistic analysis were included in the multivariate logistic model. Cut-off points were determined according to statistical and clinical perspectives. The odds ratio (OR) was calculated in conjunction with the 95% confidence interval (CI). Stata ver. 8 software (Stata Corporation, College Station, TX, USA) was used for all statistical analyses.

Results

Incidence of severe hemorrhage and patient characteristics

Of the 283 patients registered and evaluable in the JALSG APL97 study, four were excluded because of insufficient data for the purpose of the present analysis. Thus, a total of 279 patients were analyzed. They comprised 76 in Group A, 67 in Group B, 52 in Group C, 78 in Group AD, and 6 in Group BD. Severe hemorrhage during remission induction therapy occurred in 18 patients (6.5%), comprising intracranial hemorrhage in 12, pulmonary hemorrhage in 4, and both in 2 cases. Cumulative incidence at 60 d was 5.8% (95% CI: 4.8–9.0%) (Fig. 1). Baseline characteristics of patients with and without severe hemorrhage are summarized in Table 1. Patients who developed severe hemorrhage were likely to present with worse performance status, lower levels of plasma fibrinogen, and higher fibrin degradation product (FDP) ratios, which were calculated by dividing the serum FDP value by its upper normal limit. No differences in age, morphological subtype (M3 or M3v), WBC counts, or platelet counts were observed between the two groups. Table 2 shows details of those who developed severe hemorrhage, indicating that none of the cases had been treated with ATRA alone (Group A). The median duration from the start of chemotherapy to the onset of bleeding was 5 d (range, 0–17 d).

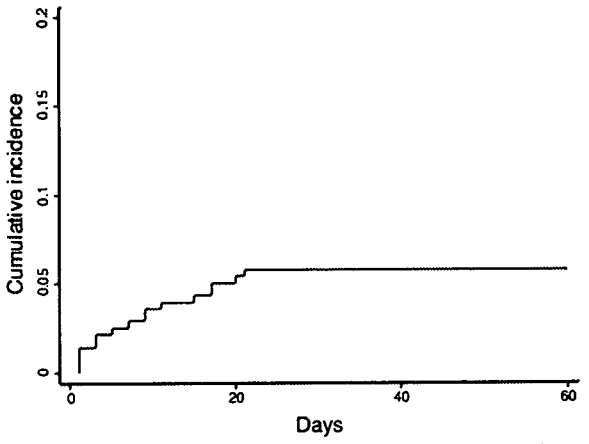

Figure 1 Cumulative incidence of severe hemorrhage. The incidence of grade 3 or higher intracranial and/or pulmonary hemorrhage during remission induction therapy was 5.8%.

At the day of onset, 71% of the patients reached the targeted level of platelet count (30×10^9/L), whereas only 40% reached the targeted level of fibrinogen (1.5 g/L).

Risk factors

Next, risk factors for severe hemorrhage were investigated. Univariate analysis disclosed that three pretreatment factors, i.e., fibrinogen level (≥ or < 1.0 g/dL), WBC count (≥ or $< 20 \times 10^9$/L), and performance status (0–1 or 2–3) were associated with severe hemorrhagic complications, all of which maintained their predictive value by multivariate analysis (Table 3). When the cut-off point of WBC count was set at 10×10^9/L, statistically significant association was not observed (P = 0.106). Also, no significant correlation was detected for age, platelet count, or FDP ratio (P = 0.896, 0.741, and 0.440, respectively). The ratios for patients who developed severe hemorrhage were 12.5% for those with fibrinogen levels below 1.0 g/dL, 15.6% for those with WBC counts exceeding 20×10^9/L, 13.7% for those with performance status of 2 or 3. Additionally, patients with sever hemorrhage were more easily prone to develop RA syndrome (OR, 3.97; 95% CI, 1.38–11.4) or pneumonia (OR, 3.27; 95% CI, 0.99–10.8) than patients without hemorrhage.

Outcome

Of the 18 patients with severe hemorrhage, nine suffered an early death at a median of 15 d (range, 1–22 d) after start of treatment. Death within 7 d occurred in three patients. Their causes of deaths comprised intracranial

Table 1 Patient characteristics

Severe hemorrhage	Absent	Present	*P*-value
No. of patients	261	18	
Age (yr)	48 (15–70)	44 (16–63)	0.837
Sex (male/female)	147/114	9/9	0.631
FAB type (M3/M3v)	243/18	18/0	0.616
Performance status (0–1/2–3)	215/44	11/7	0.003
WBC count ($\times 10^9$/L)	1.7 (0.1–151.6)	3.3 (0.6–256.5)	0.257
PB blast count ($\times 10^9$/L)	0.5 (0.0–145.6)	2.5 (0.0–252.7)	0.079
Platelet count ($\times 10^9$/L)	15 (2–238)	40 (4–128)	0.834
Fibrinogen (g/L)	1.37 (0.20–5.80)	0.96 (0.42–2.28)	0.020
FDP ratio[1]	11.1 (0.29–524)	16.6 (6.56–190)	0.014

Continuous variables are presented as median (range).
[1] Calculated by dividing the FDP value by its upper normal limit.
FAB, French-American-British; WBC, white blood cell; PB, peripheral blood; FDP, fibrin degradation product.

Table 2 Details of patients who developed severe hemorrhage during remission induction course

UPN	Induction therapy[1]	Site of bleeding	Onset of hemorrhage	Platelet count at onset ($\times 10^9$/L)	Fibrinogen level at onset (g/L)	Pneumonia	RA syndrome	Outcome	Survival (yr)
26	C	CNS	Day 3	NA	NA	–	–	Alive in CR1	6.1+
37	AD	CNS	Day 7	35	1.11	–	–	Death in CR1	2.9
46	B	CNS	Day 5	47	1.63	–	–	Death in CR1	1.0
75	C	CNS	Day 11	2	NA	–	–	Early death	
85	AD	Lung	Day 21	9	0.69	–	–	Early death	
103	C	CNS	Day 1	46	1.05	+	–	Alive in CR1	2.0+
112	B	CNS	Day 3	32	1.65	–	+	Early death[2]	
116	AD	Both	Day 9	60	2.54	+	+	Death in CR1	0.2
124	AD	Lung	Day 17	7	4.49	+	+	Early death	
125	C	Both	Day 1	42	1.27	–	–	Early death	
147	C	CNS	Day 1	108	2.70	–	–	Early death	
164	AD	Lung	Day 20	36	2.24	+	+	Alive in CR1	5.1+
167	AD	Lung	Day 17	9	0.60	–	+	Alive in CR1	5.0+
206	B	CNS	Day 0	46	0.56	–	–	Alive in CR1	4.2+
233	AD	CNS	Day 15	13	0.90	–	–	Early death	
239	C	CNS	Day 1	51	0.67	–	+	Early death	
256	B	CNS	Day 9	51	0.67	–	–	Early death	
310	AD	CNS	Day 0	66	NA	–	–	Alive in CR1	2.6+

[1] Types of induction therapies are detailed in the text.
[2] The main cause of death for this patient was RA syndrome, but in association with CNS bleeding.
RA, retinoic acid; CNS, central nervous system; NA, not assessed; CR1, first remission; UPN, unique patient number.

Table 3 Factors associated with development of severe hemorrhage

	Univariate analysis	Multivariate analysis		
	P-value	*P*-value	OR (95% CI)	Factor
Fibrinogen level	0.022	0.024	3.28 (1.17–9.19)	Lower than 1.0 g/L
			1.00	1.0 g/dL or higher
WBC count	0.033	0.029	3.61 (1.14–11.4)	20×10^9/L or higher
			1.00	Lower than 20×10^9/L
Performance status	0.026	0.045	3.04 (1.02–9.02)	2–3
			1.00	0–1

OR, odds ratio; 95% CI, 95% confidence interval; WBC, white blood cell.

hemorrhage ($n = 6$), pulmonary hemorrhage ($n = 2$), and RA syndrome ($n = 1$). All of the remaining nine patients eventually achieved CR, but two remitters died because of intracranial hemorrhage which had developed during the induction course. None of the patients died of hemorrhagic complications occurring at other sites. Figure 2 shows Kaplan-Meier curves of EFS for patients with and without severe hemorrhagic complications during the remission induction course. The 5-yr EFS rate was 68.1 ± 3.2% for those who did not suffer severe hemorrhage and 31.1 ± 11.5% for those who did ($P < 0.0001$). For patients who achieved CR, on the other hand, there was no difference in DFS between patients with and without severe hemorrhage ($P = 0.6043$, Fig. 3). Six of the seven patients who survived severe hemorrhagic complications during induction therapy were alive and disease-free after a median follow-up duration of 4.6 yr (range, 2.0–6.1 yr).

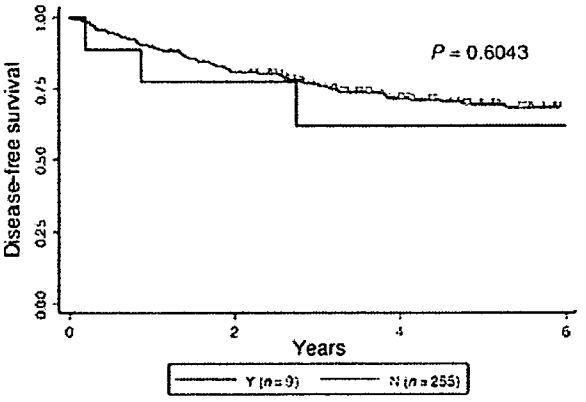

Figure 3 Probability of disease-free survival. Patients who did (Y) and did not (N) develop severe hemorrhage during remission induction therapy are compared.

Discussion

APL presents significant coagulopathy, which is occasionally exacerbated by the initiation of cytotoxic chemotherapy. In the pre-ATRA era, early hemorrhagic death reportedly occurred in up to 20% of the patients (14, 15). Recent laboratory and clinical studies have shown that ATRA produces rapid resolution of coagulopathy (16, 17), and reduces the incidence of fatal hemorrhage to a range of 2.4–11.6% (4–13). However, early hemorrhagic death remains a matter of vital concern because current treatment combining ATRA and chemotherapy induces CR in nearly all APL patients if early hemorrhagic death can be avoided, and a majority of them are to be potentially cured after the completion of standard postremission therapy (4–13). In this study, we analyzed the findings for 279 patients registered in the JALSG APL97 study, to describe severe hemorrhagic complications during induction therapy with respect to incidence, risk factors, and influence on outcome.

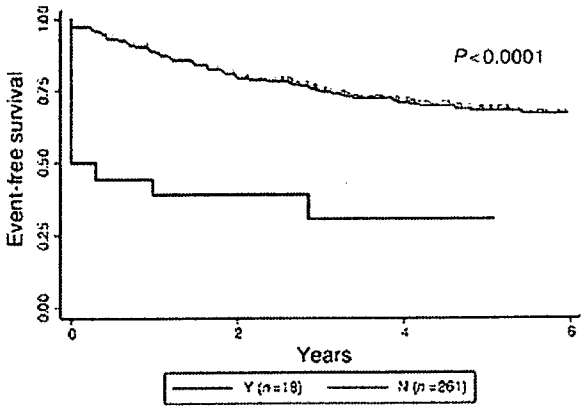

Figure 2 Probability of event-free survival. Patients who did (Y) and did not (N) develop severe hemorrhage during remission induction therapy are compared.

Severe hemorrhage developed in 6.5%, half of whom succumbed to an early death. Although direct comparisons with other studies would have some limitations particularly because of different induction regimens, the early hemorrhagic death rate of 3.2% for our patients was in the lower range of previously reported studies (Table 4). The rate in our previous study was also as low as 3.3% (11). Considering that no patient treated with ATRA alone suffered a severe hemorrhage in this study, one of the specific reasons for these findings might be the withholding of chemotherapy in a subset of patients (Group A). However, this should be interpreted with caution because eight patients in Group AD developed severe hemorrhage. Also, it should be noted that 5.8% of early hemorrhagic deaths were reported in one study in which all patients were treated with ATRA alone (6).

Although most of the patients who developed severe hemorrhage were receiving frequent transfusions, the targeted levels of platelet counts and plasma fibrinogen were reached at the day of bleeding in only 71% and 40%, respectively (Table 2). This finding indicates that for patients at high risk of hemorrhage, more intensive transfusion policy may be beneficial. The risk factors identified by our analysis: initial fibrinogen level, WBC count, and performance status, should be helpful for the assessment of high-risk patients.

As shown in Fig. 2, patients who did not experience hemorrhagic complications had an excellent long-term outcome, suggesting that early death represents the major obstacle for a cure of this disease. Although introduction of ATRA has resulted in a decrease in fatal

Authors	Induction therapy	No. of patients	No. of CR (%)	No. of EHD (%)
Fenaux *et al.* (4)	ATRA ± DNR/Ara-C	54	49 (91)	3 (5.6)
Estey *et al.* (5)	ATRA + IDR	43	33 (77)	5 (11.6)
Tallman *et al.* (6)	ATRA	172	124 (72)	10 (5.8)
Mandelli *et al.* (7)	ATRA + IDR	240	229 (95)	8 (3.3)
Fenaux *et al.* (8)	ATRA + DNR/Ara-C	413	229 (95)	10 (2.4)
Sanz *et al.* (9)	ATRA + IDR	123	381 (92)	8 (6.5)
Lengfelder *et al.* (10)	ATRA + TAD/HAM	51	47 (92)	3 (5.9)
Asou *et al.* (11)	ATRA ± DNR/BHAC	369	333 (90)	12 (3.3)
Sanz *et al.* (12)	ATRA + IDR	426	384 (90)	25 (5.9)
Schlenk *et al.* (13)	ATRA + (IDR or ICE)	82	72 (88)	6 (7.3)
Current study	ATRA ± IDR/Ara-C	279	264 (95)	9 (3.2)

Table 4 Incidence of early hemorrhagic death in various acute promyelocytic leukemia (APL) studies

CR, complete remission; EHD, early hemorrhagic death; ATRA, all-*trans* retinoic acid; DNR, daunorubicin; Ara-C, cytarabine; IDR, idarubicin; TAD, 6-thioguanine, Ara-C, DNR; HAM, high-dose Ara-C and mitoxantrone; BHAC, behenoyl Ara-C; ICE, IDR, Ara-C, and etoposide.

hemorrhage, such complications remain of major importance and efforts to prevent them should be pursued. Our findings suggest that patients who have the high-risk features may benefit from aggressive supportive care. In the ongoing JALSG APL204 study, we are aiming to further reduce the incidence of severe hemorrhagic complications by stratifying patients into three groups on the basis of risk factors identified in the present study and by prospectively applying different criteria for transfusion threshold.

Acknowledgements

This study was supported in part by the Grants for Cancer from Ministry of Health, Welfare and Labor and by the Grant for Cancer Translational Research Project from Ministry of Education, Culture, Sports, Science and Technology, Government of Japan.

References

1. Avvisati G, Lo Coco F, Mandelli F. Acute promyelocytic leukemia: clinical and morphologic features and prognostic factors. *Semin Hematol* 2001;**38**:4–12.
2. Tallman MS, Nabhan C, Feusner JH, Rowe JM. Acute promyelocytic leukemia: evolving therapeutic strategies. *Blood* 2002;**99**:759–67.
3. Grimwade D, Lo Coco F. Acute promyelocytic leukemia: a model for the role of molecular diagnosis and residual disease monitoring in directing treatment approach in acute myeloid leukemia. *Leukemia* 2002;**16**:1959–73.
4. Fenaux P, Le Deley MC, Castaigne S, *et al.* Effect of all transretinoic acid in newly diagnosed acute promyelocytic leukemia. Results of a multicenter randomized trial. European APL 91 Group. *Blood* 1993;**82**:3241–9.
5. Estey E, Thall PF, Pierce S, Kantarjian H, Keating M. Treatment of newly diagnosed acute promyelocytic leukemia without cytarabine. *J Clin Oncol* 1997;**15**:483–90.
6. Tallman MS, Andersen JW, Schiffer CA, *et al.* All-*trans*-retinoic acid in acute promyelocytic leukemia. *N Engl J Med* 1997;**337**:1021–8.
7. Mandelli F, Diverio D, Avvisati G, *et al.* Molecular remission in PML/RAR alpha-positive acute promyelo cytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood* 1997;**90**:1014–21.
8. Fenaux P, Chastang C, Chevret S, *et al.* 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* 1999;**94**:1192–200.
9. Sanz MA, Martin G, Rayon C, *et al.* A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RARalpha-positive acute promyelocytic leukemia. PETHEMA group. *Blood* 1999;**94**:3015–21.
10. Lengfelder E, Reichert A, Schoch C, *et al.* Double induction strategy including high dose cytarabine in combination with all-*trans* retinoic acid: effects in patients with newly diagnosed acute promyelocytic leukemia. German AML Cooperative Group. *Leukemia* 2000;**14**:1362–70.
11. Asou N, Adachi K, Tamura U, *et al.* Analysis of prognostic factors in newly diagnosed patients with acute promyelocytic leukemia: the APL92 study of the Japan Adult Leukemia Study Group (JALSG). *Cancer Chemother Pharmacol* 2001;**48**(Suppl 1):S65–71.
12. Sanz MA, Martin G, Gonzalez M, *et al.* Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. *Blood* 2004;**103**:1237–43.
13. Schlenk RF, Germing U, Hartmann F, *et al.* High-dose cytarabine and mitoxantrone in consolidation therapy for acute promyelocytic leukemia. *Leukemia* 2005;**19**:978–83.