prognostic factors for OS and only WBC count (30×10^9/L or higher) was an independent prognostic factor for DFS (Table 4). We developed a simple scoring system for predicting outcome based on the HR of these risk factors for OS of CR patients. A score of one was allocated to each of the following parameters: age ≥ 35 years, PS 2 or 3, WBC counts ≥ 30×10^9/L and other miscellaneous abnormal karyotype, and a score of 2 to the very high/high risk karyotype. OS curves of patients scoring 0, 1, 2, 3, and 4 or more are shown in Fig. 2c. The 5-year OS rate for patients scoring 0 was 60% (95% CI 45–73%). OS decreased with an increasing total score, and 4-year OS rate for patients scoring 4 or more was only 10% (95% CI 1–35%; Table 5).

Table 3 Summary of therapy results

	All patients	Ph-negative	Ph-positive
Patients eligible	404	256	116
Early deaths	21 (5%)	12 (5%)	9 (8%)
Refractory	85 (21%)	36 (14%)	42 (36%)
Dead	70	28	38
Alive	15	8	4
CR achievement (% of all)[a]	298 (74%)	208 (81%)	65 (56%)
Died in CR	24	14	6
Relapse[b]	170	121	38
Dead	143	99	36
Alive	27	22	2
CCR	104	73	21
Total dead	258	153	89
Total alive	146	103	27

CCR continuous complete remission, *CR* complete remission, *Ph* Philadelphia chromosome

[a] CR achievement includes those reached CR by induction therapy and 1st consolidation therapy

[b] Relapse indicates the first relapse after CR achievement including the first relapse after hematopoietic stem cell transplantation (HSCT) among those who received HSCT during CR

3.5 HSCT for Ph-negative patients

Among 208 Ph-negative patients who achieved CR, 60 (29%) underwent allo-HSCT during their first CR (37 from a related donor and 23 from an unrelated donor). The median duration from the time of achieving CR to transplantation was 7.5 months (range 3.1–34.6 months). Patients who received allo-HSCT were significantly younger than those who did not [median (range) 25.5 years (16.0–55.0) vs. 31.0 years (15.0–64.0), $P = 0.02$]. Among 60 patients who received allo-HSCT, 8 (13%) died in remission, 16 (27%) relapsed, and 36 (60%) were in continuous CR (CCR). The 5-year OS rate was 63% (95% CI 49–74%; Fig. 3a), 68% (95% CI 50–81%) from a related donor and 55% (95% CI 32–73%) from an unrelated donor, showing no significant difference ($P = 0.43$). Patients scoring 0 or 1 had significantly better OS [75% (95% CI 55–86%)] than those scoring 2 or more [48% (95% CI 26–67%)] ($P = 0.02$; Fig. 3b).

Among 148 patients who did not receive allo-HSCT during their first CR, 37 (25%) were in CCR, 6 (4%) died in remission (2, therapy-related death; one, other disease; 3, unknown) and 105 (71%) relapsed. Of 105 relapsed, 46 received allo-HSCT for salvage therapy, and 10 were alive in remission after transplantation with a median duration of 3.9 years (range 7 months to 7.1 years). The 5-year OS

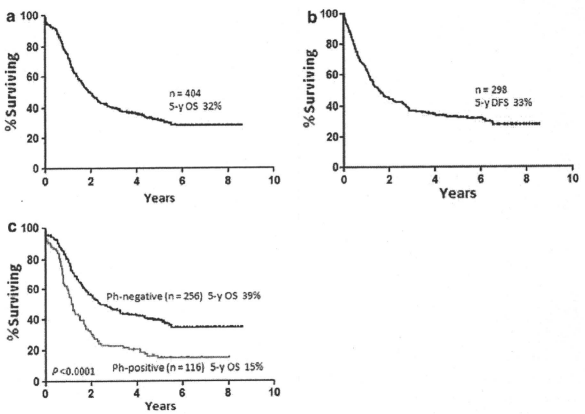

Fig. 1 Survival analysis. **a** Overall survival (OS) of 404 eligible patients. **b** Disease-free survival (DFS) of 298 patients who achieved complete remission. **c** OS of 116 Philadelphia chromosome (Ph)-positive patients and 256 Ph-negative patients

Table 4 Effects of clinical and biological features on outcome among Ph-negative ALL (univariate analyses)

Parameters	No. of patients at diagnosis	CR		OS		DFS	
		%	*P*	No. of events[a]	Hazard ratio (95% CI)	No. of events[b]	Hazard ratio (95% CI)
Total	256	81		153		135	
Sex							
Female	136	85	0.08	82	Ref	80	Ref
Male	120	77		71	1.08 (0.78–1.48)	55	0.86 (0.60–1.23)
Age (years)							
15–24	98	85	0.72	52	Ref	50	Ref
25–34	43	79		23	0.93 (0.57–1.52)	22	0.96 (0.57–1.61)
35–54	70	80		44	1.31 (0.87–1.96)	36	1.06 (0.68–1.65)
55 or older	45	78		34	1.87 (1.21–2.90)	27	1.37 (0.84–2.21)
Performance status							
0, 1	230	83	0.03	133	Ref	123	Ref
2, 3	26	65		20	2.00 (1.25–3.21)	12	1.56 (0.84–2.90)
Hepatomegaly							
No	198	81	0.74	111	Ref	99	Ref
Yes	58	83		42	1.50 (1.05–2.15)	36	1.71 (1.15–2.53)
Splenomegaly							
No	207	83	0.12	120	Ref	110	Ref
Yes	49	74		33	1.29 (0.88–1.91)	25	1.37 (0.88–2.14)
Lymphadenopathy							
No	176	89	0.30	106	Ref	94	Ref
Yes	80	78		47	0.98 (0.69–1.39)	41	1.13 (0.77–1.65)
Fever over 38°C							
No	178	81	0.83	104	Ref	96	Ref
Yes	78	82		49	1.12 (0.80–1.58)	39	0.84 (0.57–1.24)
CNS involvement							
No	253	82	0.09	150	Ref	134	Ref
Yes	3	33		3	3.01 (0.96–9.46)	1	1.40 (0.20–9.99)
WBC count ($\times 10^9$/L)							
Less than 30	185	86	0.002	105	Ref	97	Ref
30 or higher	71	69		48	1.66 (1.17–2.33)	38	1.80 (1.22–2.65)
Immunologic classification							
B-lineage	199	84	0.14	115	Ref	107	Ref
T-lineage	35	74		22	1.20 (0.76–1.92)	19	1.30 (0.78–2.17)
Chromosome category (n = 236), unknown = 20							
Standard risk	9	89	0.49	3	Ref[c]	3	Ref[c]
Normal	121	79		65		58	
Miscellaneous	64	78		44	1.68 (1.14–2.46)	35	1.47 (0.95–2.26)
High risk	10	90		5	1.87 (1.21–2.89)[c]	5	1.82 (1.15–2.89)[c]
Very high risk	32	91		25		24	
Days from treatment start to CR achievement							
≤30 days	85			44	Ref	54	Ref
>30 days	120			66	1.00 (0.68–1.46)	78	1.02 (0.71–1.46)

ALL acute lymphoblastic leukemia, *CNS* central nervous system, *CR* complete remission, *DFS* disease-free survival, *OS* overall survival, *Ph* Philadelphia chromosome

[a] Death

[b] Relapse or death

[c] The standard-risk group was combined with the normal karyotype group, and the high risk group with the very high risk group

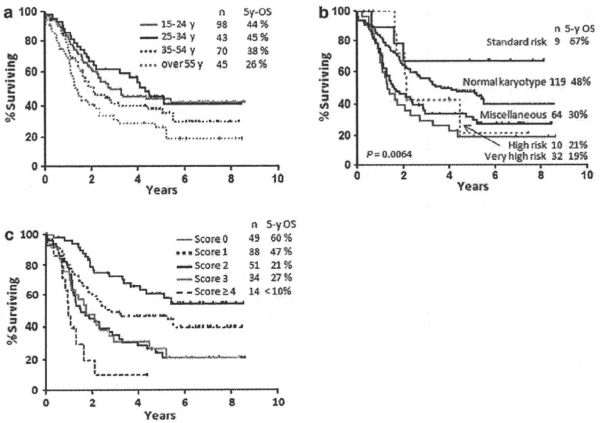

Fig. 2 Survival analysis of Philadelphia chromosome-negative patients. **a** Overall survival (OS) by age group. **b** OS by karyotype category according to the modified MRC UKALLXII/ECOG E2993ALL cytogenetic subgroups: the very high risk group included t(4;11), complex karyotype defined as more than 5 abnormalities without known translocations, or low hypodiploidy/near triploidy; the high risk group included other *MLL* translocations, monosomy 7 with less than 5 abnormalities or t(1;19); the standard-risk group included high hyperdiploidy; other miscellaneous abnormal karyotypes were categorized as intermediate risk. **c** OS by a scoring system that we developed. A score of one was allocated to each of the following parameters; age $\geq$ 35 years, performance status 2 or 3, WBC counts $\geq 30 \times 10^9$/L and other miscellaneous abnormal karyotype, and a score of 2 to the very high/high risk karyotype

Table 5 Effects of clinical and biological features on survival among Ph-negative ALL (multivariate analyses)

Parameters	HR (95% CI)		
	OS	OS of CR patients	DFS
Age (years old)			
35 or older (vs. 15–34)	1.74 (1.24–2.44)	1.64 (1.11–2.43)	1.21 (0.83–1.74)
Performance status			
2, 3 (vs. 0, 1)	2.06 (1.26–3.37)	1.94 (1.02–3.69)	1.43 (0.74–2.77)
Hepatomegaly			
Yes (vs. no)	1.26 (0.86–1.85)	1.43 (0.91–2.23)	1.44 (0.94–2.21)
WBC count ($\times 10^9$/L)			
30 or higher (vs. less than 30)	1.42 (0.98–2.01)	1.16 (0.73–1.82)	1.63 (1.08–2.48)
Chromosome category			
Miscellaneous group (vs. SR + NK[a])	1.55 (1.05–2.29)	1.56 (0.98–2.50)	1.26 (0.81–1.97)
High and very high risk (vs. SR + NK[a])	1.60 (1.02–2.50)	2.25 (1.37–3.70)	1.49 (0.92–2.41)

ALL acute lymphoblastic leukemia, *CR* complete remission, *DFS* disease-free survival, *HR* hazard ratio, *OS* overall survival, *Ph* Philadelphia chromosome

[a] Standard risk + normal karyotype

rate for all Ph-negative patients who did not receive allo-HSCT during the first CR was 37% (95% CI 29–46%; Fig. 3c). Among those, the 5-year OS rate for patients scoring 0 or 1 was 53% (95% CI 41–63%) and that for patients scoring 2 or more was 19% (95% CI 10–31%), showing a significantly better OS in the former than the latter ($P < 0.0001$; Fig. 3d).

3.6 HSCT for Ph-positive patients

Among 65 Ph-positive patients who achieved CR, 22 (34%) underwent allo-HSCT during their first CR (19 from a related donor and 3 from an unrelated donor). The median duration from the time of achieving CR to transplantation was 4.6 months (range 2.6–12.1 months).

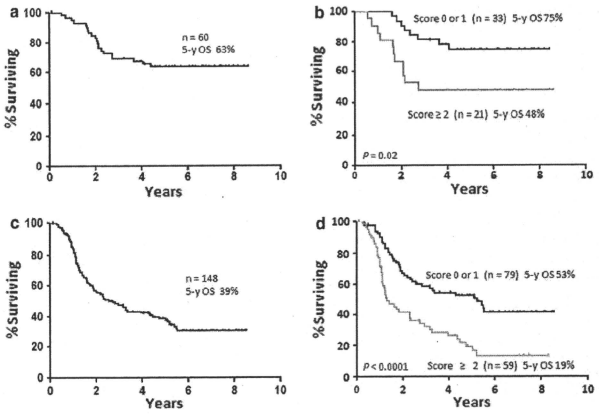

Fig. 3 Survival analysis of Philadelphia chromosome-negative patients with/without allogeneic-hematopoietic stem cell transplantation (allo-HSCT) in first complete remission. **a** Overall survival (OS) in those who received allo-HSCT. **b** OS in those who received allo-HSCT by dichotomized prognostic score group. **c** OS in those who did not received allo-HSCT. **d** OS in those who did not received allo-HSCT by dichotomized prognostic score group

Patients who received allo-HSCT were significantly younger than those who did not [median (range) 41.5 years (15–56) vs. 49.0 years (24–63), $P = 0.02$]. Among 22 Ph-positive patients who received allo-HSCT, 5 (23%) died in remission, 6 (27%) relapsed, and 11 (50%) were in CCR. The 5-year OS rate was 47% (95% CI 24–67%; Fig. 4).

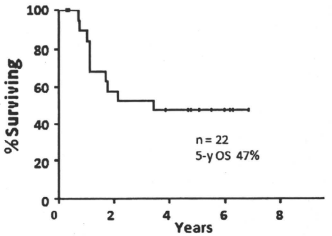

Fig. 4 Overall survival (OS) of Philadelphia chromosome-positive patients who received allo-hematopoietic transplantation in first complete remission

4 Discussion

In the present study, although the CR rate of all 404 evaluable patients did not exceed 80%, the rate was greater in Ph-negative patients (81%) than Ph-positive patients (56%). These results are not so different from our preceding JALSG-ALL93 study [4] (Ph-negative, 83%; Ph-positive, 51%) and from the CALGB 8811 study [12] (Ph-negative, 84%; Ph-positive, 70%). In the JALSG-ALL93 study, we tested an intensified induction therapy mainly using DOX. In the present study, we asked whether a benefit could be achieved by intensifying the consolidation phase of the CALGB 8811 study protocol, mainly using DOX. However, DFS of CR patients did not differ much from that of the CALGB 8811 study or that of the CALGB 9111 study [3] in which the same chemotherapy regimen was used. Besides, the 5-year OS of 45% for Ph-negative patients who achieved CR was similar to that in the MRC UKALL XII/ECOG E2993 study [7], suggesting that the present intensified consolidation therapy resulted in a similar outcome to the standard consolidation regimen, and had little impact on the survival improvement of adult Ph-negative ALL.

Age is a major prognostic factor in ALL. When we compared by age, OS of patients younger than 35 years was significantly better than that of patients aged 35 years or older (5-year OS; 44 vs. 32%, $P = 0.008$); however, there was no significant difference between patients of 15–24 and 25–34 years old. A similar outcome was seen in the MRC UKALL XII/ECOG E2993 study [7], i.e., the 5-year OS rates for Ph-negative patients aged 15–19 and 20–29 years old were 44 and 45%, respectively.

Several retrospective analyses reported improved outcomes for adolescent and young adult ALL treated by the pediatric regimens [20, 21]. Stock et al. [21] reported the outcomes of 321 adolescents and young adults who underwent pediatric (Children's Cancer Group) or adult (CALGB) trials, and the 7-year OS rates were 67 and 46%, respectively. As one potential explanation for these differences, they suggested dose intensification of nonmyelosuppressive drugs, such as glucocorticoids, VCR and

L-ASP, which have been the mainstay of pediatric ALL therapy. The outcome of adolescents and young adults in our study was similar to that of the same cohort in the CALGB study, including the 8811 trial, and we did not use L-ASP during the post-remission therapy. Therefore, to improve the therapeutic outcome of adult ALL, particularly that of adolescent and young adult ALL, pediatric regimens using dose-intensified nonmyelosuppressive drugs should be prospectively tested. Such studies are already underway in several adult cooperative study groups, including the JALSG-202 study, showing promising results [22, 23].

The outcome of T-ALL patients in JALSG-ALL97 study has previously been reported together with T-ALL patients in other JALSG ALL studies [24]. Reportedly, the T-cell phenotype is generally a favorable prognostic factor in adult ALL; however, the outcome of T-ALL patients in our present study was not better than that of Ph-negative precursor B-ALL. T-ALL was said to be benefited from Ara-C and CPM [25]. In our consolidation phase, high doses of anthracycline and CPM were used, but not Ara-C. Thus, T-ALL may not have been benefitted from anthracycline in consolidation therapy. T-ALL therapy may need a higher dose of Ara-C and/or a new drug such as nelarabine, a promising drug for T-cell malignancies [26, 27].

In the present study, we were able to confirm the impact of cytogenetics on the outcome of adult ALL based on the grouping by MRC UKALL XII/ECOG E2993 study [10] and SWOG 9400 study [11]. In addition to Ph, the very high risk group in the present study was t(4;11), complex type and low hypodiploidy/near triploidy, and the outcome (5-year OS, 19%) of this group was very similar to the SWOG 9400 study (22%) and the MRC UKALL XII/ECOG E2993 study (22–28%), suggesting that this grouping is useful for the prediction of poor prognostic group. Normal diploidy is the most frequent karyotype among Ph-negative ALL. In the present study, the 5-year OS rate of patients with a normal karyotype was 48%, which was similar to that of the MRC UKALL XII/ECOG E2993 study (48%) and the SWOG 9400 study (50%). In contrast, the prognosis of other miscellaneous types was worse in the present study than in the SWOG 9400 study. This group includes numerous cytogenetic abnormalities, and the prognostic risk of each type has not been defined because the number of each type is very small. In fact, in the MRC UKALL XII/ECOG E2993 study, the largest study of adult ALL, most other miscellaneous types did not show any significant association with disease outcome, and only a few karyotypes exceeded 45% 5-year OS, showing no conflict to our results. Since the high risk group in the present study, comprising other *MLL* translocations, monosomy 7 or t(1;19), showed a poor prognosis, we combined this group with the very high risk group for statistic analysis, although the outcome of the high risk group in the SWOG 9400 study was not particularly detrimental. It seems difficult to discuss the difference because of the small number of patients in each study (SWOG study, 12 patients vs. present study, 10).

In our previous JALSG-ALL93 study, CR patients under 40 years old with human leukocyte antigen-matched siblings were scheduled to receive allo-HSCT during the first CR. In this study, however, we did not incorporate recommendation for HSCT except for patients with Ph or t(4;11), because the ALL93 study showed no survival difference between patients of age under 40 years with and without a sibling donor, except for Ph-positive patients who benefited from allo-HSCT. However, if patients without a sibling wished to have HSCT, most of them can obtain an unrelated donor through the Japan Marrow Donor Program. Approximately 30% of Ph-negative patients who achieved CR underwent allo-HSCT in their first CR, and 38% of them from unrelated donors. The 5-year OS rate in Ph-negative patients who received allo-HSCT during the first CR was 63% and the transplantation-related mortality rate was only 13%. Notably, the 5-year OS of patients without risk factors, such as older age, advanced PS, a higher WBC count and unfavorable karyotypes, was 75% and very satisfactory despite of marked selection bias in the choice of treatment. Recently, the MRC/ECOG group reported that matched related allo-HSCT for adult ALL in the first CR provided survival benefit for standard-risk patients in prospective sibling donor versus no-donor comparison [28]. The HOVON Cooperative Group also stated that standard-risk ALL patients showed favorable survival following allo-HSCT, due to both a strong reduction of relapse and a modest transplantation-related mortality, although their standard-risk criteria did not include age [29]. These results suggest that allo-HSCT is the most promising treatment modality for adult ALL patients who have achieved CR and have few risk factors.

Multivariate Cox analysis in our Ph-negative patients showed that older age (35 years old or more), advanced PS (PS 2 or 3) and unfavorable karyotypes (very high/high risk or other miscellaneous abnormalities) were independent adverse prognostic factors for OS, and a higher WBC count (30×10^9/L or more) for DFS. The 5-year OS of patients without these risk factors was 60%, whereas that of patients with multiple risk factors was under 30%. Our scoring system worked well for both patients who received HSCT or did not in their first CR. This demonstrates importance to assess prognostic factors, including cytogenetics, when making a treatment plan. Further studies on this scoring system should be performed to prove its usefulness in the individualized therapy on Ph-negative ALL possessing different prognostic scores.

Regretfully, the present study could not show the benefit of intensified consolidation with myelosuppressive drugs in

adult ALL. Dose intensification of nonmyelosuppressive agents such as glucocorticoids, VCR and L-ASP like pediatric regimens and/or incorporation of new agents such as molecule-targeting drugs and monoclonal antibodies would be the next step to be tested in order to increase the cure rate of adult ALL.

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Correlation Between Imatinib Pharmacokinetics and Clinical Response in Japanese Patients With Chronic-Phase Chronic Myeloid Leukemia

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Despite the outstanding results generally obtained with imatinib mesylate (IM) in the treatment of chronic myeloid leukemia (CML), some patients show a poor molecular response. To evaluate the relationship between steady-state trough plasma IM concentration (IM-C_{min}) and clinical response in CML patients, we integrated data from six independent Japanese studies. Among 254 CML patients, the mean IM-C_{min} was 1,010.5 ng/ml. Importantly, IM-C_{min} was significantly higher in patients who achieved a major molecular response (MMR) than in those who did not ($P = 0.002$). Multivariate analysis showed that an MMR was associated with both age (odds ratio (OR) = 0.97 (0.958–0.995); $P = 0.0153$) and with IM-C_{min} (OR = 1.0008 (1.0003–1.0015); $P = 0.0044$). Given that patients with IM-C_{min} values >1,002 ng/ml had a higher probability of achieving an MMR in our large cohort ($P = 0.0120$), the data suggest that monitoring of IM levels in plasma may improve the efficacy of IM therapy for CML patients.

Imatinib mesylate (IM) is a potent and selective inhibitor of the BCR-ABL tyrosine kinase and the autophosphorylation of the tyrosine kinase receptor c-KIT, and it has been approved for the treatment of Philadelphia chromosome–positive chronic myeloid leukemia (CML)[1] and gastrointestinal stromal tumors.[2] Despite the outstanding results generally achieved with IM in CML, there have been cases of treatment failure, as well as cases in which the response to IM was suboptimal.[3] Factors that might be associated with suboptimal responses to IM include (i) biological factors, such as the baseline presence or later emergence of BCR-ABL mutations and other genetic variants; (ii) clinical features, such as the disease status of the patient or the Sokal risk score at baseline; (iii) pharmacokinetics-related interindividual pharmacogenetic variations and/or drug–drug interactions affecting IM metabolism; and (iv) adherence.[4–6]

Several previous studies have investigated whether variations in the concentration of IM in plasma influence the clinical response of IM-treated patients; however, the studies produced varied results (for a summary, see **Table 1**).[6–12] For example, data from three studies suggested a correlation between the trough plasma IM concentration (IM-C_{min}) and clinical response among CML patients.[6–8] Larson *et al.* reported that the IM-C_{min} was significantly higher in patients who achieved a complete cytogenetic response (CCyR) than in patients without a CCyR.[7] In addition, Picard *et al.* reported that an IM-C_{min} of 1,002 ng/ml should be set as an efficacy threshold because this concentration was significantly associated with a major molecular response (MMR) in 68 chronic-phase CML patients.[8] In contrast, Forrest *et al.* did not find any correlation between IM-C_{min} and clinical response among 78 CML patients after a minimum of 12 months of IM therapy.[9] However, as stated by the authors, their results

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Table 1 Correlation of imatinib pharmacokinetics with clinical response

Authors	Disease	No. of patients	IM daily dose (mg)	IM-C_{min} (ng/ml)	Correlation with response
Picard *et al.*[8]	CML	50	400	1,058 ± 557	Yes (CCyR, MMR)
		18	600	1,444 ± 710	
Larson *et al.*[7]	CML	351	400	979 ± 530	Yes (CCyR)
Forrest *et al.*[9]	CML	78	400	999 (203–2,910)	No (CCyR, MMR)
Widmer *et al.*[12]	CML	20	400	NA	No (AUC_u vs. HR)
	GIST	38	600	NA	Yes (AUC_u vs. OR)
Kawaguchi *et al.*[10]	CML	13	400	1,400 ± 570	NA
		9	300	1,150 ± 440	
Demetri *et al.*[11]	GIST	36	400	1,530 ± 666	Yes (TTP, OOBR)
		37	600	1,752 ± 794	
Marin *et al.*[6]	CML	84	400	900 (400–1,600)	Yes (MMR)

AUC_u, free area under the curve; CCyR, complete cytogenetic response; CML, chronic myeloid leukemia; GIST, gastrointestinal stromal tumor; HR, hematologic response; IM, imatinib mesylate; MMR, major molecular response; NA, not available; OOBR, overall objective benefit rate; OR, overall response; TTP, time to progression.

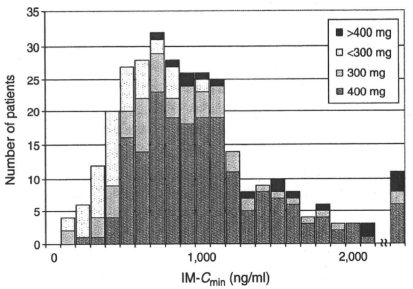

Figure 1 Distribution of steady-state trough plasma IM concentration (IM-C_{min}; $n = 314$, data obtained from all doses). The mean and median values of IM-C_{min} were 1,010.5 ± 564.6 and 900 ng/ml (range, 111–3,620 ng/ml), respectively.

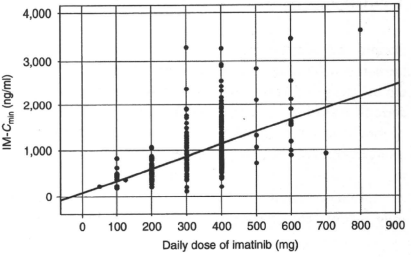

Figure 2 Steady-state trough plasma IM concentration (IM-C_{min}) achieved with the indicated daily dosages ($n = 314$). The IM-C_{min} predicted using linear regression analysis was related to dose as follows: 76.29 + 2.624 × dose ($r^2 = 0.232$; $P < 0.00001$).

may have been influenced by sample size and heterogeneous sampling times.

In Japan, the relationship between IM-C_{min} and clinical response has been independently studied by six groups.[13–18] Although two of these studies identified a significant correlation between IM-C_{min} and clinical response,[13,17] the others did not; however, these latter four studies that detected no correlation may have been insufficiently powered because of their modest sample sizes. In this study, our aim was to investigate the usefulness of monitoring IM-C_{min} in a large cohort of CML patients by integrating the data from these six Japanese studies.

RESULTS

Data for 314 Japanese patients with chronic-phase CML (189 men and 125 women) were integrated in this analysis. The median age of the patients was 60 years (range, 16–91 years), the mean body weight was 61.3 ± 12.1 kg (median, 60.0 kg; range, 37–103 kg), and the mean body surface area was 1.64 ± 0.19 m^2 (median, 1.65 m^2; range, 1.19–2.25 m^2). Among the study participants, 190 (60.5%) received 400 mg of IM daily, 59 (18.8%) received 300 mg, 48 (15.3%) received <300 mg, and 17 (5.4%) received >400 mg. The median duration of IM therapy was 1,435 days (range, 56–2,582 days).

The distribution of IM-C_{min} values across all doses is shown in **Figure 1**. The mean and median IM-C_{min} values were 1,010.5 ± 564.6 and 900 ng/ml (range, 111–3,620 ng/ml), respectively. Although there was substantial interpatient variability among patients treated with the same dose of IM, IM-C_{min} increased significantly and proportionately across doses ranging from 50 to 800 mg (**Figure 2**). Of these 314 patients, 60 patients were excluded from further analysis aimed at correlating IM-C_{min} with clinical response because the duration of IM therapy was <12 months or because a molecular response was not evaluated. The clinical characteristics of the 254 patients included are summarized in **Table 2**. There were no correlations between IM-C_{min} and age, body weight, body surface area, or the duration of IM therapy ($P = 0.343$, $P = 0.073$, $P = 0.075$, and $P = 0.931$, respectively), gender (Student's *t*-test: $P = 0.648$), or Sokal risk score (analysis of variance: $P = 0.399$).

Table 2 Association between potential predictive factors and imatinib trough concentration

Variable (n = 254)	Mean or no. of patients	Correlation with imatinib concentration (r)	P-value
Quantitative features[a]			
Age (years)	59.2	0.060	0.343
Weight (kg)	61.1	−0.113	0.073
Body surface area (m^2)	1.6	−0.112	0.075
Duration of IM therapy (days)	1,454.2	−0.005	0.931
Qualitative features			
Sex (male/female)[b]	151/103		0.648
Sokal risk group (low/intermediate/high)[c]	91/91/52		0.399

IM, imatinib mesylate.

[a]Compared using Pearson's product-moment correlation analysis. Data are presented as correlation coefficient (r) or mean values. [b]Compared using Student's t-test. [c]Compared using analysis of variance.

Table 3 Patient characteristics and clinical response to imatinib therapy

Characteristic (no. of patients)	MMR (166)	No MMR (88)	P-value	CCyR (218)	No CCyR (36)	P-value
Quantitative features						
Imatinib concentration (ng/ml)	1,107.4 ± 594.4	872.7 ± 528.5	0.002	1,057.8 ± 585.0	835.0 ± 524.3	0.033
Age (years)	57.1 ± 15.4	62.8 ± 14.2	0.004	58.3 ± 15.2	64.3 ± 14.4	0.029
Body weight (kg)	61.1 ± 12.1	61.5 ± 12.0	0.808	61.1 ± 12.0	61.6 ± 12.5	0.818
Body surface area (m^2)	1.643 ± 0.195	1.645 ± 0.188	0.926	1.638 ± 0.200	1.643 ± 0.191	0.888
Daily imatinib dose (mg)	367.5 ± 79.6	323.6 ± 135.4	0.006	365.1 ± 94.0	272.0 ± 127.0	0.0002
Duration of imatinib therapy (days)	1,459 ± 623	1,458 ± 697	0.986	1,450 ± 643	1,482 ± 705	0.786
Qualitative features						
Sex (male/female)	95/71	56/32	0.349	128/90	23/13	0.572
Sokal risk group (low/intermediate/high)	63/61/33	28/30/19	0.779	81/81/43	10/11/9	0.529

Data are presented as mean values (±SD) for quantitative features. Quantitative variables were compared using Student's t-test. Qualitative variables were compared using the χ^2 or Fisher's exact test.

CCyR, complete cytogenetic response; MMR, major molecular response.

Among all the patients evaluated, 166 (65.3%) achieved an MMR, and 218 (85.8%) achieved a CCyR (**Table 3**). IM-C_{min} values were significantly higher in patients with an MMR than in those without an MMR; the mean values were 1,107.4 ± 594.4 ng/ml (median, 986 ng/ml) and 872.7 ± 528.5 ng/ml (median, 719.5 ng/ml), respectively (P = 0.002). In addition, there were significant differences in age and daily dosage between patients with an MMR and those without an MMR (P = 0.004 and 0.006, respectively). Importantly, when we subclassified all the patients according to their IM-C_{min} as previously reported,[8] we found that patients with an IM-C_{min} ≥1,002 ng/ml had a higher probability of achieving an MMR than those with an IM-C_{min} <1,002 ng/ml (P = 0.0120, **Figure 3**).

In addition, IM-C_{min} was significantly higher in patients with a CCyR (n = 219) than in those without a CCyR (n = 36); the mean values were 1,057.8 ± 585.0 ng/ml (median, 916 ng/ml) and 835.0 ± 524.3 ng/ml (median, 688 ng/ml), respectively (P = 0.033). There were also significant differences in age and daily dosage between the group with a CCyR and those without a CCyR (P = 0.029 and 0.0002, respectively, **Table 3**).

In a stepwise forward-selection multiple logistic analysis, MMR was associated with both the age of the patient (odds ratio (OR) = 0.97 (0.958–0.995); P = 0.0153) and the IM-C_{min} value

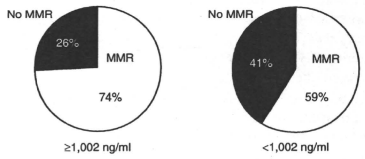

Figure 3 Correlation of trough plasma IM concentration (IM-C_{min}) with MMR (n = 254). IM-C_{min} values ≥1,002 ng/ml had a significantly higher probability of achieving an MMR (Fisher's exact test; P = 0.0120). MMR, major molecular response.

(OR = 1.0008 (1.0003–1.0015); P = 0.0044), whereas a CCyR was associated with only daily dosage (OR = 1.0073 (1.0036–1.0110); P = 0.0001). The association between IM-C_{min} and CCyR was not observed in the stepwise forward-selection multiple logistic analysis.

DISCUSSION

IM has favorable pharmacokinetic characteristics, including complete bioavailability and a proportionate dose–response relationship.[4,19] However, we found that, although there was a

linear relationship between IM-C_{min} and the daily dose of IM, there was also substantial interpatient variability. Factors that could underlie this interpatient variability include body size, age, gender, liver function, renal function, interaction with other medications given concomitantly, adherence to medication regimens, and polymorphisms of enzymes or transporters related to IM pharmacokinetics and/or pharmacodynamics. In this analysis, we did not observe any correlation between IM-C_{min} and body weight, body surface area, or age. Moreover, the eligibility criteria of each of the integrated studies ensured that there were no patients with serious renal or hepatic dysfunction, and no patients who were taking other drugs that might interact with IM. In addition, adherence to medication regimens was monitored by self-report for at least 7 days prior to blood sampling.

In our study of 254 evaluated IM-treated CML patients, steady-state IM-C_{min} correlated significantly with both MMR and CCyR. Among those who achieved an MMR, the mean IM-C_{min} (1,107.4 ng/ml from all doses, 1,154.3 ng/ml from 400 mg) was >1,002 ng/ml, previously shown to be an effective IM-C_{min} threshold.[8] Moreover, we found that patients with an IM-C_{min} <1,002 ng/ml had a lower probability of achieving an MMR ($P = 0.0120$, Figure 3), thereby supporting the previously reported 1,002 ng/ml plasma concentration efficacy threshold for Japanese CML patients.

Prior to data integration, two of the six individual studies revisited in this report found a statistically significant difference in plasma IM concentrations between patients with an MMR ($n = 34$) and those without an MMR ($n = 28$) ($P = 0.010$ by Student's t-test)[13] and between patients with an optimal response ($n = 25$) and those with a suboptimal or failed response ($n = 8$) ($P = 0.0087$ by Student's t-test).[17] In the other four Japanese studies, it is possible that the number of patients per study was too small to achieve a statistically significant correlation between IM-C_{min} and outcome, as was previously suggested by Forrest *et al.*[9]

Our results suggest that higher IM-C_{min} is associated with an increased likelihood of achieving MMR and CCyR ($P = 0.002$ and 0.033, respectively, in univariate analysis). Additionally, in a multivariate analysis, both IM-C_{min} and the age of the patient were independently predictive of achieving an MMR. Age is one of the clinical factors that is included in the Sokal risk score as a baseline characteristic. Although we could not find a significant difference in Sokal risk score between patients with an MMR and those without, previous studies have reported that the Sokal risk score predicts outcome for IM-treated CML patients.[9,20] In contrast, by multivariate analysis, daily dosage of IM was the only independent predictive factor for achieving a CCyR. Together, these findings suggest that variability in IM exposure has clinical implications and, probably more so, implications for a molecular response.

Several clinical trials have established that a molecular response is a surrogate marker for predicting the likelihood of disease progression and/or survival in patients with CML.[20] In this study, although we could not directly compare IM-C_{min} with long-term outcomes, higher IM-C_{min} was associated with an increased likelihood of achieving an MMR. Given that an MMR may indicate a decreased risk for progression to accelerated phase or blastic crisis, we speculate that increased IM-C_{min} would be associated with longer survival; however, further study is necessary to test this hypothesis.

Given the importance of the duration of IM treatment in an evaluation of clinical response,[20] 60 of the 314 patients (19%) were excluded from further analysis of the correlation between IM-C_{min} and clinical response, either because the duration of IM therapy was <12 months or because a molecular response was not evaluated. Although there was no significant difference in C_{min} values between the excluded patients ($n = 60$; 994.5 ± 481.9 ng/ml) and the included patients ($n = 254$; 1,026.1 ± 582.2 ng/ml) per Student's t-test ($P = 0.315$), a potential source of bias cannot be entirely ruled out in this retrospective analysis.

In conclusion, on the basis of our data, we propose that, in addition to BCR-ABL mutation analysis for CML patients, it may be useful to assay plasma IM levels when making decisions related to IM therapy. Further study is necessary to prospectively confirm the link between IM-C_{min} and clinical response, including survival, in large multiethnic patient populations.

METHODS

Patients. The 314 patients included in this analysis were those who had previously been enrolled in six independent Japanese clinical studies.[13–18] All the patients had Philadelphia chromosome (Ph)-positive chronic-phase CML, and all were treated orally with IM for >2 months. Informed consent was obtained from each participant in accordance with the Declaration of Helsinki. Each study protocol was reviewed and approved by the ethics committees or institutional review boards of the participating centers.

Clinical parameters including response to the therapy. A CCyR was defined as the absence of Ph^+ metaphase cells among 20 or more bone marrow cells examined. In some cases, fluorescent *in situ* hybridization was also carried out for detection of bcr-abl fusion genes in neutrophils from peripheral blood.[21] An MMR was defined as a threefold log reduction in bcr-abl transcripts measured using real-time reverse transcriptase–mediated quantitative PCR and/or AMP-CML. The samples used to evaluate IM response and those for measurement of IM-C_{min} were collected from patients on the same day.

Measurement of IM concentrations in plasma. Blood samples were collected by venipuncture 24 h (± 2 h) after oral administration of IM. Plasma was isolated by centrifugation at 1,900*g* for 15 min and stored at −40 °C until analysis. IM-C_{min} values were determined using high-performance liquid chromatography coupled to electrospray-ionization tandem mass spectrometry[22] at the TORAY Research Center, (Nihonbashi, Tokyo, Japan), which is the only assay system in Japan authorized by Novartis Global.

Statistical analyses. Statistical analyses were carried out using SPSS statistical software (version 17.0; SPSS Japan, Tokyo, Japan). Data are presented as mean values ± SD unless indicated otherwise. Pearson's product moment correlation was applied to assess the relationship between IM-C_{min} and clinical variables (age, body weight, body surface area, and duration of IM therapy). A linear regression analysis was applied to assess the correlation between IM-C_{min} and the daily dose of IM. Differences in IM-C_{min} between two patient groups were evaluated using the Student's t-test. Comparison of IM-C_{min} among three groups was made using one-way analysis of variance with *post hoc* Tukey's multiple-comparison procedure. The χ^2 test or Fisher's exact test was used to compare the proportions of patients with an MMR or a CCyR and to compare groups. Stepwise forward-selection multiple logistic analyses

were performed for MMR and CCyR in order to determine the effects of the factors examined in the univariate analysis. Values of $P < 0.05$ were considered significant.

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CONFLICT OF INTEREST

The authors declared no conflict interest.

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ORIGINAL ARTICLE

Pre-transplant imatinib-based therapy improves the outcome of allogeneic hematopoietic stem cell transplantation for *BCR–ABL*-positive acute lymphoblastic leukemia

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A high complete remission (CR) rate has been reported in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) following imatinib-based therapy. However, the overall effect of imatinib on the outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is undetermined. Between 2002 and 2005, 100 newly diagnosed adult patients with Ph + ALL were registered to a phase II study of imatinib-combined chemotherapy (Japan Adult Leukemia Study Group Ph + ALL202 study) and 97 patients achieved CR. We compared clinical outcomes of 51 patients who received allo-HSCT in their first CR (imatinib cohort) with those of 122 historical control patients in the pre-imatinib era (pre-imatinib cohort). The probability of overall survival at 3 years after allo-HSCT was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and 44% (95% CI, 35–52%) for the pre-imatinib cohort. Multivariate analysis confirmed that this difference was statistically significant (adjusted hazard ratio, 0.44, $P=0.005$). Favorable outcomes of the imatinib cohort were also observed for disease-free survival ($P=0.007$) and relapse ($P=0.002$), but not for non-relapse mortality ($P=0.265$). Imatinib-based therapy is a potentially useful strategy for newly diagnosed patients with Ph + ALL, not only providing them more chance to receive allo-HSCT, but also improving the outcome of allo-HSCT.
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Introduction

The Philadelphia chromosome (Ph) presents in 20–25% of adult patients with acute lymphoblastic leukemia (ALL) and is an extremely unfavorable prognostic factor. The outcome of patients with Ph-positive ALL (Ph + ALL) following conventional chemotherapy is dismal, showing <20% long-term survival.[1–4] Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) has offered a curative option in Ph + ALL,[3–5] relatively high rates of relapse and non-relapse mortality (NRM) impair the treatment success even after allo-HSCT. The International Bone Marrow Transplant Registry reported a leukemia-free survival rate of 38% following human leukocyte antigen (HLA)-identical allo-HSCT for Ph + ALL patients transplanted in the first complete remission (CR).[6] Previously, we and others reported that imatinib-based chemotherapy produced very high CR rate, thus allowing a high proportion of patients to prepare for allo-HSCT.[7,8] However, because of the short observation period, the impact of imatinib-based therapy upon the survival outcomes after allo-HSCT remains unclear. To address whether allo-HSCT after imatinib-based therapy is a superior treatment approach to that after conventional chemotherapy, we conducted a retrospective analysis of Ph + ALL patients who underwent allo-HSCT before and after imatinib became available, using data from the Japan Adult Leukemia Study Group (JALSG) Ph + ALL202 study and from the nationwide database of the Japan Society of Hematopoietic Stem-cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP).

Patients and methods

Data source and patient selection criteria

We compared the transplantation outcome of patients treated by the JALSG Ph + ALL 202 study (imatinib cohort) with those in the historical control data in the pre-imatinib era from the JSHCT and JMDP (pre-imatinib cohort), in which information on patient survival, disease status and long-term complications, including chronic graft-versus-host disease (cGVHD) and second malignancies, is renewed annually using follow-up forms.[9,10] To

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attain an adequate level of comparability in terms of allo-HSCT, patients were selected according to the following criteria: (1) patients with *de novo* Ph+ALL; (2) age range of 15–65 years and (3) allo-HSCT during their first CR. A total of 122 patients who received allo-HSCT between January 1995 and December 2001 (before the approval of imatinib by the Japanese government) were selected. This study period of the pre-imatinib cohort included the pioneering period of cord blood transplantation (CBT) when the relevance of cell dose and HLA matching had not yet been recognized. Thus, the subjects were limited to those who received bone marrow (BM) or peripheral blood (PB) as a treatment graft.

Patients

Between September 2002 and May 2005, 100 newly diagnosed patients with Ph+ALL were registered to the JALSG Ph+ALL202 study, and received a phase 2 imatinib-combined chemotherapy as described previously.[7] Ph+ALL was diagnosed by the presence of Ph through chromosome and/or FISH analysis, and positivity for *BCR–ABL* fusion transcripts detection by real-time quantitative polymerase chain reaction (RQ–PCR) analysis.

Of 97 patients who achieved CR, 60 patients received allo-HSCT in their first CR. Of these 60 patients, 9 patients who received unrelated CBT were excluded in this analysis because of the reason as described at the selection criteria for control patients in the pre-imatinib era. Thus, 51 patients transplanted between February 2003 and December 2005 were analyzed. In the JALSG Ph+ALL202 study, allo-HSCT was recommended after achieving CR if an HLA-identical donor was available. The stem cell source for allo-HSCT was chosen in the following order: (1) matched-related allo-HSCT; (2) HLA-A, B and DRB1 allele matched (6/6) or DRB1 one-allele mismatched-unrelated allo-BMT, if patients had no HLA-matched-related donor and (3) unrelated CBT or HLA-mismatched-related allo-HSCT, if they had no donors described in (1) and (2). A prophylaxis for GVHD was determined by each institute, but did not include T-cell depletion. The study was approved by the institutional review board of each participating center and conducted in accordance with the Declaration of Helsinki.

Definition of engraftment and GVHD

Engraftment day was defined as the first day of three consecutive days when the absolute neutrophil count was $\geqslant 0.5 \times 10^9$/l. Graft failure was defined as the lack of any sign of neutrophil recovery. Engraftment that occurred after day 60 was also considered to be a graft failure. Patients who died early (<day 29) were excluded from the analysis of engraftment. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined according to previously described standard criteria.[11]

Quantitation of BCR–ABL transcripts

The copy number of *BCR–ABL* transcripts in BM was determined at a central laboratory using the RQ–PCR as described previously.[7] To minimize the variability in the results because of differences in the efficiency of cDNA synthesis and RNA integrity among the patient samples, the copy number of the *BCR–ABL* transcripts was converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The normalized values of the *BCR–ABL* copies in each sample were reported as *BCR–ABL* number of copies. At least 5.7×10^5 copies/μg RNA *GAPDH* levels were required in a sample to consider a negative PCR result valid; otherwise, the sample was not useful for minimal residual studies. The threshold for quantification was 50 copies/μg RNA. The levels below this threshold were designated as 'not detected' or '<50 copies/μg'. In this study, the former was categorized as PCR negativity.

Minimal residual disease (MRD) at the time of HSCT was evaluated by the result of RQ–PCR within 30 days prior to transplantation.

Statistical considerations

The primary end point of this study was overall survival (OS) after allo-HSCT. Secondary end points included disease-free survival (DFS) and the incidence of aGVHD, cGVHD, NRM and relapse. We defined DFS events as relapse or death, whichever occurred earlier. The observation periods for OS were calculated from the date of transplantation until the date of the event or last known date of follow-up. The probabilities of OS and DFS were estimated using the Kaplan–Meier product limit method. The cumulative incidences of NRM, relapse, aGVHD and cGVHD were estimated as described elsewhere, taking the competing risk into account.[12] In each estimation of the cumulative incidence of an event, death without an event was defined as a competing risk. Risk factors for OS and DFS were evaluated by a combination of uni- and multivariate analyses. The following variables were evaluated for each analysis: imatinib-based therapy prior to HSCT, age group (under 40 versus 40 to 54 versus 55 and older), stem cell source (BM versus PB), HLA disparity (matched (HLA-identical siblings or 6/6 allele matched unrelated) versus mismatched), duration from diagnosis to HSCT and cGVHD as time-varying covariate (yes versus no). Univariate analysis was performed using Cox regression models or log-rank test. Multivariate analysis was performed using Cox proportional hazards regression model or competing risk regression model[13] as appropriate. For the evaluation of time-varying events, such as aGVHD or cGVHD, upon clinical outcomes, we treated these as time-varying covariates. Differences among groups in terms of demographic characteristics were tested using the χ^2 or Mann–Whitney tests as appropriate. All statistical analyses were conducted using STATA 11 (STATA Corp., College Station, TX, USA).

Results

Patient characteristics

In the imatinib cohort, there were 29 males and 22 females, with a median age of 38 years (range, 15–64 years). Regarding transcript types, 36 patients had minor *BCR–ABL* and 15 had major *BCR–ABL*. In 5 patients, pre-treatment cytogenetic data were not available, and of the remaining 46 patients, 8 showed t(9;22) only, 36 had additional chromosome aberrations and 2 showed normal karyotype. Of 48 patients who were evaluable for MRD analysis, 36 patients achieved PCR negativity at the time of HSCT.

Some of the clinical and biological features (such as presence of additional chromosome aberrations, *BCR–ABL* subtype, MRD status at HSCT and performance status at HSCT) were not available in the pre-imatinib cohort and not included in the present analysis.

Table 1 lists the characteristics of patients included in this comparative analysis. Some of the clinical features were significantly different between two cohorts: age distribution at HSCT ($P=0.048$), conditioning regimens ($P<0.001$), GVHD prophylaxis ($P<0.001$) and duration from diagnosis to HSCT ($P=0.041$). The majority of patients received the preparatory

Table 1 Patient characteristics ($N=173$)

Characteristic	*Imatinib cohort*	*Pre-imatinib cohort*	P
No. of transplantations	51	122	
Age, n (%)			0.048
–39	27 (53)	71 (58)	
40–54	17 (33)	49 (40)	
55–	7 (14)	2 (2)	
Median (range)	38 (15–64)	38 (15–57)	
Gender (male/female)	29/22	73/49	0.717
HSCT donor, n (%)			0.460
Related	24 (47)	73 (60)	
Unrelated	21 (41)	43 (35)	
HLA-mismatched related	6 (12)	6 (5)	
Hematopoietic cell source, n (%)			0.246
Bone marrow	35 (69)	94 (77)	
Peripheral blood	16 (31)	28 (23)	
Conditioning regimen, n (%)			<0.001
CY+TBI	24 (47)	26 (22)	
CY+CA+TBI	14 (27)	37 (31)	
CY+VP+TBI	2 (4)	21 (17)	
CY+TESPA+TBI	—	7 (6)	
CY+BU+TBI	—	6 (5)	
Flu+BU	3 (6)	—	
Flu+ LPAM ± TBI	2 (4)	—	
Others	6 (12)	25 (20)	
GVHD prophylaxis, n (%)			<0.001
Cyclosporine + sMTX	24 (47)	95 (80)	
Cyclosporine ± other	3 (6)	3 (2)	
Tacrolimus + sMTX	22 (43)	17 (14)	
Tacrolimus + other	—	4 (3)	
Median days from diagnosis to HSCT (range)	162 (67–512)	182 (66–834)	0.041

Abbreviations: BU, oral busulfan; CA, cytarabine; CY, cyclophosphamide; Flu, fuludarabine; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; LPAM, melphalan; sMTX, short-term methotrexate; TBI, total body irradiation; TESPA, tespamine; VP, etoposide.

regimen of total body irradiation followed by cyclophosphamide and/or cytarabine. Five patients aged >55 in the imatinib cohort were given a reduced intensity regimen consisting of fludarabine and melphalan or busulfan. In the pre-imatinib cohort, a combination of cyclosporine (CsA) and short-term methotrexate (sMTX) was mostly used in the prophylaxis of GVHD. On the other hand, both CsA + sMTX and tacrolimus (FK506) + sMTX combinations were commonly used in the imatinib cohort. In both cohorts, none of the patients received imatinib therapy after HSCT in their first CR. In the imatinib cohort, all patients who showed hematologic relapse after HSCT received salvage treatment comprising of imatinib and/or chemotherapy. As for the pre-imatinib cohort, 13 patients relapsed after the approval of imatinib by the Japanese government (beyond December 2001). However, we have no information on how many patients received imatinib-based therapy after their relapse. The median follow-up period for survivors was 2.6 years (range, 1.0–4.6 years) for the imatinib cohort and 6.9 years (range, 0.1–11.4 years) for the pre-imatinib cohort.

Outcome

OS and DFS. In the pre-imatinib cohort, 80 patients died after HSCT: 46 of disease recurrence and 34 of causes other than leukemia. In the imatinib cohort, 35 patients were alive, 32 of them were free of leukemia and 16 patients died after HSCT: 4 of disease recurrence and 12 of causes other than leukemia. The 3-year OS was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and significantly higher than 44% (95% CI, 35–52%) for the pre-imatinib cohort ($P=0.0148$; Figure 1a). The 3-year DFS was 58% (95% CI, 41.8–70.9%) for the imatinib cohort and significantly higher than 37% (95% CI, 28.5–45.6%) for the pre-imatinib cohort ($P=0.039$; Figure 1b).

Table 2 shows the result of risk factor analysis for OS and DFS among all 173 patients. In the multivariate analysis, the only variable found to influence OS and DFS was the pre-transplant imatinib-based therapy (hazard ratio (HR)=0.44 (95% CI, 0.25–0.77); $P=0.004$ and HR=0.51 (95% CI, 0.31–0.86); $P=0.011$, respectively). The presence of cGVHD showed a tendency of favorable OS and DFS, but did not reach the statistical significance (HR=0.66 (95% CI, 0.42–1.06); $P=0.085$ and HR=0.75 (95% CI, 0.47–1.19); $P=0.217$, respectively).

Other outcomes of transplantation

Relapses. In the pre-imatinib cohort, 48 patients relapsed after HSCT with a median of 240 days (range, 42–2302 days).

In the imatinib cohort, 7 patients (3 of 36 with PCR negative and 4 of 12 with PCR positive at HSCT) relapsed after HSCT with a median of 137 days (range, 68–728 days). The estimated cumulative incidence of relapse at 3 years was 15.0% (95% CI, 6.6–26.7%), and significantly lower than that of the pre-imatinib cohort (50.4% at 3 years (95% CI, 39.6–60.2%); $P=0.002$; Figure 1c). Among patients in the imatinib cohort, patients with PCR negative showed significantly lower relapse rate compared with that of PCR positive (10.0% (95% CI, 2.5–23.6%) versus 41.3% (95% CI, 16.9–64.4%) at 3 years, respectively, $P=0.025$).

Non-relapse mortality. In the pre-imatinib cohort, 34 patients died of non-relapse causes at a median of 159 days (range, 5–2094 days) after HSCT. The estimated cumulative incidence of NRM in the pre-imatinib cohort was 28% (95% CI, 20–36) at 3 years (Figure 2a). In the imatinib cohort, 12 patients died of non-relapse causes at a median of 329 days (range, 41–850 days) after HSCT. The 3-year cumulative incidences of NRM were 21% (95% CI, 11–33%; Figure 2a). There were no significant differences between two cohorts ($P=0.265$).

Cause of death. Recurrence of the primary disease was the leading cause of death in both groups: 55% for the pre-imatinib cohort and 25% for the imatinib cohort. In the pre-imatinib cohort, the causes of NRM were organ failure (11%), infection (9%), GVHD (8%), transplantation-associated thrombotic microangiopathy (TMA) (4%), interstitial pneumonia (3%), graft failure (3%) and other causes (6%). In the imatinib cohort, the causes of NRM included infection (19%), bronchiolitis obliterans with organizing pneumonia (13%), TMA (13%), GVHD (13%), organ failure (6%) and other causes (12%).

Graft-versus-host disease. There was no significant difference in the cumulative incidence of Grades 2–4 aGVHD between two cohorts (31% (95% CI, 19–44%) versus 37% (95% CI, 29–46%), $P=0.391$; Figure 2b). The cumulative incidence of cGVHD at 1 year after HSCT was significantly higher in the imatinib cohort than in the pre-imatinib cohort (49% (95% CI, 31–64%) versus 27% (95% CI, 18–37%), $P=0.0261$; Figure 2c).

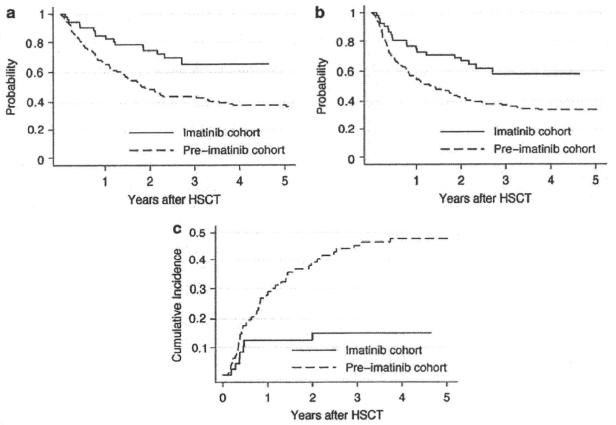

Figure 1 Transplantation outcomes of 51 patients who received imatinib-based therapy and 122 historical patients. (**a**) Overall survival, (**b**) disease-free survival and (**c**) cumulative incidence of relapse.

Table 2 Results of uni- and multivariate analysis of overall survival and disease-free survival among 173 patients with Ph+ALL

Characteristic	*Overall survival*				*Disease-free survival*			
	Univariate analysis		*Multivariate analysis*		*Univariate analysis*		*Multivariate analysis*	
	RR (95% CI)	P	*RR (95% CI)*	P	*RR (95% CI)*	P	*RR (95% CI)*	P
Imatinib-imterim therapy before HSCT	0.45 (0.26–0.77)	0.004	0.44 (0.25–0.77)	0.004	0.51 (0.31–0.83)	0.007	0.51 (0.31–0.86)	0.011
Donor status (RE versus UR)	0.87 (0.57–1.32)	0.521	0.72 (0.40–1.30)	0.275	0.77 (0.51–1.16)	0.211	0.65 (0.37–1.16)	0.147
Age at HSCT (–39 versus 40–55 versus 55–)	1.03 (0.74–1.44)	0.852	1.12 (0.78–1.62)	0.536	0.98 (0.71–1.36)	0.914	1.03 (0.73–1.47)	0.862
HLA-disparity (matched versus mismatched)	0.90 (0.39–2.06)	0.800	0.76 (0.32–1.81)	0.531	1.11 (0.49–2.54)	0.800	1.06 (0.45–2.50)	0.895
Stem-cell source (BM versus PB)	1.15 (0.72–1.82)	0.565	1.23 (0.72–2.10)	0.451	1.30 (0.85–2.00)	0.228	1.34 (0.81–2.20)	0.254
Days from diagnosis to HSCT	1.00 (0.99–1.00)	0.217	1.00 (0.99–1.00)	0.141	1.00 (0.99–1.00)	0.415	1.00 (0.99–1.00)	0.125
cGVHD as time-varying covariate (yes versus no)	0.68 (0.43–1.08)	0.101	0.66 (0.42–1.06)	0.085	0.78 (0.50–1.23)	0.292	0.75 (0.47–1.19)	0.217

Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; CI, confidence interval; cGVHD, chronic graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hemtopoetic stem cell transplantation; PB, peripheral blood; Ph, Philadelphia chromosome; RE, related; RR, relative risk; UR, unrelated.

However, regarding the cumulative incidence of extensive-type cGVHD, there was no significant difference between two cohorts (22% (95% CI, 10–36%) versus 12% (95% CI, 6–20%), $P=0.119$; Figure 2d).

Association between cGVHD and OS/DFS/relapse. To examine the difference of impacts of cGVHD upon clinical outcome in the pre- and imatinib cohorts, we conducted stratified analysis by cohort, treating cGVHD as a time-varying covariate (Table 3). Multivariate analysis revealed that, in the imatinib cohort, there were no significant associations between cGVHD and OS/DFS/relapse ($P=0.707$, 0.332 and 0.713, respectively). On the other hand, in the pre-imatinib cohort, there was a significant association between cGVHD and OS (HR = 0.59 (95% CI, 0.35–1.00), $P=0.048$), but not between cGVHD and DFS/relapse ($P=0.234$ and 0.338, respectively).

Engraftment. In the pre-imatinib cohort, three patients experienced graft failure. The median periods to reach the neutrophil count of $>0.5\times10^6$/l and platelet count of 50×10^6/l were 15 days (range, 8–49 days) and 25 days (range, 9–120 days), respectively, for evaluable patients. In the imatinib cohort, all 51 patients were engrafted. The median period to reach a neutrophil count of $>0.5\times10^6$/l and platelet count of 50×10^9/l was 15 days (range, 5–41 days) and 25 days (range, 11–504 days), respectively, for evaluable patients. There was no

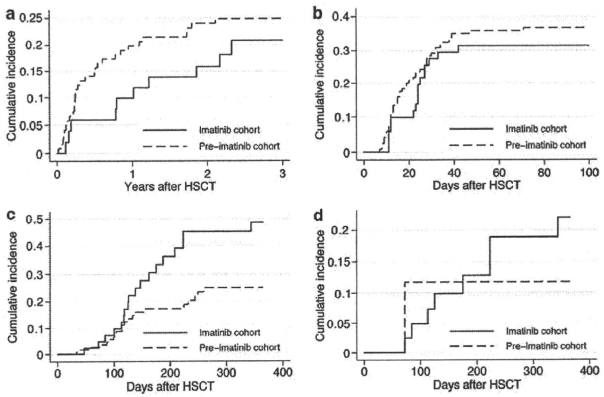

Figure 2 Cumulative incidence of GVHD or NRM. (**a**) Non-relapse mortality, (**b**) Grade 2–4 acute GVHD, (**c**) chronic GVHD and (**d**) extensive-type chronic GVHD.

Table 3 Impact of overall cGVHD on OS, DFS and relapse in multivariate analysis using cGVHD as a time-varying covariate

Cohort	*OS*			*DFS*			*Relapse*		
	Relative risk	*95% CI*	P	*Relative risk*	*95% CI*	P	*Relative risk*	*95% CI*	P
Imatinib cohort	0.80	(0.26–2.51)	0.707	0.59	(0.21–1.71)	0.332	0.74	(0.15–3.67)	0.713
Pre-imatinib cohort	0.59	(0.35–1.00)	0.048	0.73	(0.43–1.23)	0.234	0.75	(0.39–1.44)	0.388

Abbreviations: CI, confidence interval; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; HLA, human leukocyte antigen; OS, overall survival; PBSC, peripheral blood stem cell.
Data were adjusted for age categories, donors from unrelated subjects, HLA-matching status, PBSC graft and days to transplantation. Cox proportional hazard models were applied to OS and DFS, and a competing risk regression model was applied to relapse.

significant difference in neutrophil and platelet recovery between two cohorts ($P=0.201$ and 0.783, respectively).

Discussion

This study showed that patients with Ph + ALL who achieved CR by imatinib-based therapy and subsequently received allo-HSCT in their first CR showed significantly superior survival outcome to those in the pre-imatinib era. To our knowledge, our current report is the first to describe the superiority of imatinib-based therapy for this disease by analyzing a substantial number of patients with sufficient follow-up period. The treatment of Ph + ALL has changed dramatically since the introduction of imatinib and > 90% of patients have achieved CR,[7,14,15] and allows SCT to be performed in a substantial proportion of patients in major or complete molecular remission.[8,16–18] Actually, in the imatinib cohort, 97 of 100 patients (97%) achieved CR and 60 (60%) could receive allo-HSCT in their first CR. Several studies reported improved OS rates compared with that in the pre-imatinib era by incorporation of imatinib-based therapy.[14,15,19,20] However, there had been few reports focusing on the clinical impact of pre-transplant imatinib administration on the outcome of HSCT. Lee *et al*.[8] reported superior outcome of HSCT by imatinib-based therapy compared with the historical control data, in which 29 patients with prior imatinib treatment showed better outcomes in terms of relapse, DFS and OS than the historical control patients. However, their comparative analysis included patients who received HSCT for refractory disease or beyond their first CR (4 of 29 patients in the imatinib group and 16 of 33 patients in the historical group). Several studies showed that remission status at the time of HSCT was one of the most important prognostic factors for outcome.[21,22] Therefore, we contend that it would be better to assess a greater number of patients and exclude patients with advanced stage at HSCT to accurately compare the clinical impact of imatinib-based therapy on the outcome of HSCT. To our knowledge, this study has the largest number of Ph + ALL patients receiving allo-HSCT in their first CR with the longest follow-up duration yet reported.

It is noteworthy from our findings that a lower rate of relapse was found in the imatinib cohort. Our results thus suggest that an imatinib-based therapy provides a survival benefit for newly diagnosed Ph + ALL patients by lowering the rate of subsequent relapse after HSCT. Despite the lack of comparative data of MRD in the pre-imatinib cohort, 75% of patients in the imatinib cohort achieved RQ-PCR negativity for *BCR/ABL* at the time of HSCT. Moreover, the relapse rate was significantly lower among

patients with PCR negative. From these, we believe that a powerful anti-leukemia activity of the imatinib-based therapy mostly contributed to the prevention of subsequent relapse after HSCT in the present analysis. Thinking of the reduced relapse rate after HSCT, impact of cGVHD should also be considered. Several studies in the pre-imatinib era reported beneficial impact of cGVHD on relapse incidence and survival.[23–25] In this study, the incidence of cGVHD was significantly higher in the imatinib cohort compared with that in the pre-imatinib cohort. In the imatinib cohort, more patients received PB as a stem cell source, which might have contributed to the high frequency of cGVHD. Besides, longer leukemia-free survival period in the imatinib cohort might have contributed to the increased frequency of cGVHD, which is a late complication often observed in the recipients of allo-HSCT who had survived without disease for at least 3 months after transplantation. One could argue that this observation could be related to a stronger graft versus leukemia effect and contribute to the lower relapse rate. However, the presence of cGVHD had no significant impact on the OS/DFS/relapse rate in our imatinib cohort by multivariate analysis.

To assist the proper interpretation of our current results, the strengths and limitations need to be considered. As discussed earlier, one of the strengths of this study is the large sample size for the imatinib cohort, which gives us a better estimation of the end points and also adds statistical power to the analyses. In addition, adjustments for potential confounders in the comparisons with the pre-imatinib cohort from a nationwide registry allow unbiased estimates to be made, at least in Japan. Given the evidence for a substantial impact of imatinib in Ph+ALL patients,[7,14–16] it is unrealistic to conduct a prospective study comparing treatments with or without imatinib. Hence, a retrospective cohort design could be suboptimal to address the key questions.

One of the possible limitations of our current analysis could be the presence of residual confounding factors both of known and unknown. Among the known factors, a difference in the conditioning regimens could be noted. The City of Hope National Medical Center reported a favorable result from the use of a fractionated TBI–etoposide regimen in the treatment of Ph+ALL.[26] However, in the comparative analysis, the clinical advantage of this approach seemed to be established mostly among patients transplanted in their second CR.[27] Moreover, this approach was commonly applied in our pre-imatinib cohort rather than in the imatinib cohort (22 and 4%, respectively). Differences in GVHD prophylaxes should also be considered. Tacrolimus was more frequently used in the imatinib cohort than in the pre-imatinib cohort, which reflects the change in practice within the field of allo-HCT in Japan as tacrolimus was widely used for unrelated allo-HSCT since 2000. Nevertheless, the lack of any differences in the incidence of aGVHD between two cohorts indicates that this factor had minimal impact in our analysis.

It may be argued that the improved outcome of the imatinib cohort have been influenced by the pre-transplant chemotherapy in the JALSG Ph+ALL 202 study. Although detailed information on the pre-transplant chemotherapy in the pre-imatinib cohort was not available, it was clear that the majority of patients were most likely treated by the JALSG ALL93 or JALSG ALL97 protocols as pre-transplant chemotherapy,[2] as these were widely used regimens in Japan at the time. The chemotherapeutic regimen in the JALSG Ph+ALL202 study was similar to those used in these protocols. Thus, the effectiveness on Ph+ALL would have been similar between the two cohorts. At least in JALSG, there had been neither remarkable progress in the chemotherapy of Ph+ALL until the clinical introduction of imatinib, nor in other groups including the MD Anderson Cancer Center.[28] Thus, in the present analysis, the influence of pre-transplant chemotherapy appears to be quite limited.

The difference of transplant year between the two cohorts (1995–2001 and 2002–2005, respectively) could have affected the outcome of HSCT, and the improvement of transplantation procedure might have contributed to the favorable outcome in the imatinib cohort. However, Nishiwaki *et al.*[29] analyzed the clinical outcome of 641 Japanese patients with Ph-negative ALL who had received allo-HSCT in their first CR in 1993–1997, 1998–2002 and 2003–2007, and reported that there was no statistical difference in OS and NRM between three periods. In this study, the incidence of NRM was lower in the imatinib cohort, but did not reach the statistical significance. Therefore, the influence of transplantation year is thought to be limited in this study.

Considering potential benefit by imatinib, the lack of information about post-transplant imatinib use in the pre-imatinib cohort might have led us to underestimate the difference between two cohorts.

In conclusion, we have found that there is a significant improvement in the OS and DFS of Ph+ALL patients who received allo-HSCT following imatinib-based therapy. Although further validation using larger cohorts from different populations is essential to confirm our findings, imatinib-based therapy is likely to be a useful strategy for not only giving patients with Ph+ALL more chance to receive allo-HSCT, but also for improving their outcome after allo-HSCT.

Conflict of interest

Dr Naoe has received research funding and honoraria from Novartis Japan. Dr Ohnishi has received research funding from Novartis Japan. Dr Miyazaki has received honoraria from Novartis Japan. The remaining authors declare no conflict of interest.

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High expression of 67-kDa laminin receptor relates to the proliferation of leukemia cells and increases expression of GM-CSF receptor

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Objective. **The 67-kDa laminin receptor (LR) is a nonintegrin receptor for laminin, a major component of the extracellular matrix. To elucidate the role of LR in leukemia cells, we studied the relationship between the phenotype of leukemia cells and LR expression.**

Materials and Methods. **The relationship between clinical features of acute myeloid leukemia and expression of LR was examined. LR was overexpressed or suppressed by the introduction of complementary DNA or small interfering RNA for LR in a human leukemia cell line to test the effect of LR on the phenotype of leukemia. Expression of granulocyte-macrophage colony-stimulating factor receptors (GM-CSFR) was also tested in leukemia cells, including clinical samples.**

Results. **Expression of LR was significantly related to elevation of white blood cell count, lactate dehydrogenase, and survival among acute myeloid leukemia patients. Forced expression of LR enhanced proliferation, cell-cycle progression, and antiapoptosis of leukemia cells associated with phosphorylation of a transcription factor, signal transducer and activator of transcription 5, in the absence of stimulation by laminin. On the other hand, suppression of LR expression had the opposite effects. The number of GM-CSFR increased in leukemia cells overexpressing LR, and there was a significant relationship between the expression of LR and GM-CSFR in acute myeloid leukemia samples.**

Conclusions. **These results suggest that LR expression influenced the characteristics of leukemia cells toward an aggressive phenotype and increased the number of GM-CSFR. These changes might be partly related to enhanced GM-CSF signaling.** © **2011 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

Proliferation and differentiation of hematopoietic cells are strictly regulated via intrinsic and extrinsic signals [1]. Signal from the extracellular matrix (ECM), one of the extrinsic signals, has a significant influence on the control of normal and abnormal hematopoiesis [2,3]. For example, for proliferation and maintenance of leukemia-initiating cell, which is capable of propagating full-blown leukemia, a specific environment called "niche" is required, in which ECM plays a role [4–6]: stimulation from stromal cells through CD44 and its ligand [7,8] and that from the extracellular matrix through very late antigen 4 and fibronectin [9].

Laminin belongs to a family of heterotrimetric glycoproteins composed of α, β, and γ chains, which are major components of ECM [10,11]. There are >12 laminin isoforms that target multiple receptors on the cell surface. The functions of laminin are widely divergent and include the following: structural roles in the basement membrane, adhesion of normal and malignant cells to the matrix, promotion of malignant phenotypes, regulation of growth and metastasis of tumors, and induction of apoptosis through, for example, the Rho and phosphatidylinositol 3 kinase/Akt signaling

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