tively. Both the SH2 and SH3 domains are required to maintain the SRC family kinases in an inactive state: the SH2 domain binds to the C-terminal tyrosine residue in a phosphorylation-dependent manner, and the SH3 domain interacts with a short polyproline type II helix located between the SH2 domain and the kinase domain (Schindler et al., 1999; Xu et al., 1999; Young et al., 2001). These intramolecular interactions are believed to lock the molecule in a closed, inactive state, resulting in repression of kinase activity. In this regard, disruption of this closed conformation would activate the SRC family kinases and lead to cell transformation. In fact, some deletions or mutations in either the SH2 or the SH3 domain of SRC have been shown to activate its catalytic and/or transforming activities (Hirai and Varmus, 1990). Thus, the disruption of the SH3 and SH2 domains in ETV6/FRK may contribute to deregulation of kinase activity. Secondly, in the ETV6/FRK fusion protein, the entire PNT domain of ETV6 is fused to the kinase domain of FRK. As is the case with other ETV6/TK fusion proteins (Carroll et al., 1996; Golub et al., 1996; Jousset et al., 1997), the PNT domain would force dimerization of the ETV6/FRK protein and lead to constitutive tyrosine autophosphorylation and activation of the ETV6/FRK kinase.

The downstream signaling pathway mediated by ETV6/FRK still remains to be elucidated. The wild type FRK is expressed primarily in epithelial tissues (Cance et al., 1994), but also weakly in various hematopoietic cell line (data not shown). However, its functions or downstream signaling pathways remain largely unknown, especially in hematopoietic systems. The only known candidate endogenous downstream component of FRK is the SH2-domain adaptor protein SHB. According to recent reports, GTK, a rodent homologue of FRK, induces neurite outgrowth in PC12 cells and insulin stimulated signaling pathways in pancreatic insulin-producing cells via SHB (Anneren et al., 2000; Anneren and Welsh, 2002). In the present study, however, immunoblotting analysis failed to detect expression of the SHB protein in ETV6/FRK-expressing cells (data not shown). Thus, involvement of SHB in transformation by ETV6/FRK remains unclear. We also tested the phosphorylation status of several signaling molecules, including signal transducer and activator of transcription (STAT1, STAT3, STAT5, STAT6, extracellular signal-regulated kinase 1/2 (ERK1/2), P38 mitogen-activated protein kinase (P38 MAPK), phosphatidylinositol 3-kinase (PI3K), and phospholipase C (PLC)-gamma, in ETV6/FRK-expressing cells. However, we failed to detect any aberrant phosphorylation of these molecules in ETV6/FRK-expressing cells in comparison to FRK-expressing cells (data not shown). Future identification of the target substrate of ETV6/FRK might provide a novel insight into the mechanism of ETV6/FRK-induced transformation as well as of wild-type FRK-mediated signal transduction.

Finally, we demonstrated that ETV6/FRK had a dominant-negative effect over ETV6-mediated transcriptional repression. Because ETV6/FRK retains the PNT oligomerization domain of ETV6, ETV6/FRK may interfere with the transcriptional repression activity of ETV6 by heterodimerizing with wild-type ETV6. Our results indicate that ETV6/FRK is a novel oncoprotein with dual functions: deregulated tyrosine kinase activity and a dominant-negative modulation of transcriptional repression by ETV6. Because wild-type ETV6 appears to have tumor-suppressive activity (Romparey et al., 2000), its suppression by ETV6/FRK also could contribute to oncogenesis. It may be possible that ETV6/FRK can contribute to oncogenesis through two independent mechanisms: activation of the ETV6/FRK tyrosine kinase, which would lead to aberrant stimulation of the downstream signaling pathway, and inhibition of the tumor-suppressive functions of ETV6. This model suggests potential strategies for reversion of transformation by ETV6/FRK. Because the kinase-inactive mutant of ETV6/FRK is nontransforming, a specific inhibitor of the SRC family kinases may inhibit transformation by ETV6/FRK. Alternatively, overexpression of wild-type ETV6 also would interfere with the ability of ETV6/FRK to transform cells. Further experiments will explore these possibilities.

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A Prospective Trial to Evaluate the Safety and Efficacy of Pravastatin for the Treatment of Refractory Chronic Graft-Versus-Host Disease

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This prospective study evaluates the safety and efficacy of pravastatin for the treatment of chronic graft-versus-host disease (GVHD). We included 18 patients with refractory chronic GVHD. Oral pravastatin was started at 10 mg/day, and the dose was increased up to 40 mg/day in 4 weeks. This maximum dose was administered over 8 weeks. There were no severe adverse events caused by pravastatin. A clinical response was observed in the skin score in two patients, mouth score in five patients, eye score in two patients, liver score in three patients, platelet count score in one patient, and weight loss in two patients. The overall response rate was 28%. Immunophenotypic analyses showed that T-helper (Th)1 cells were dominant in all but one patient before treatment and that the Th1/Th2 ratio tended to be lower in the responders than in the nonresponders. A randomized controlled trial is warranted to evaluate the efficacy of pravastatin against chronic GVHD.

Keywords: Chronic graft-versus-host disease, pravastatin, treatment.

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Chronic graft-versus-host disease (GVHD) is one of the major complications after allogeneic hematopoietic stem-cell transplantation and develops in 25% to 80% of allogeneic transplant recipients (*1–3*). Corticosteroids and cyclosporine are most widely used to treat chronic GVHD, but they have demonstrated limited efficacy.

Pravastatin is a lipid-lowering agent that inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Recently, the immunosuppressive effect of statins has been highlighted in both clinical and laboratory studies. Pravastatin reduced the incidence of graft rejection after cardiac and kidney transplantation (*4, 5*). Statins also prevented islet allograft rejection in a mouse model (*6*). Two distinct molecular mechanisms of the immunosuppressive effect of statins have recently been proposed. First, statins suppress the induction of major histocompatibility complex-II expression by interferon-gamma on human endothelial cells and macrophages (*7*). Second, statins selectively inhibit the molecular association between leukocyte function antigen-1 and intercellular adhesion molecule-1 (*8*). With these data, we performed a prospective clinical trial to evaluate the safety and efficacy of pravastatin for the treatment of chronic GVHD.

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Patients aged between 20 and 70 years who had refractory pathologically proven chronic GVHD were eligible for the study. Refractory chronic GVHD was defined as chronic GVHD that was not improved by first-line treatment with corticosteroids at more than 0.5 mg/kg or cyclosporine at a therapeutic blood level for at least 2 weeks, or that showed progression during the tapering of first-line treatment. Patients had to demonstrate good hepatic and renal function as defined by serum bilirubin less than 85.5 μmol/L (5 mg/dL), alanine aminotransferase less than 500 IU/L, and serum creatinine less than 176.8 μmol/L (2.0 mg/dL). Patients with myopathy or who were receiving fibrates were excluded to avoid rhabdomyolysis. All of the patients provided their written informed consent. This study was approved by the institutional review board at each participating institution.

Pravastatin was started orally at 10 mg/day. The dose was increased to 20 mg/day after 2 weeks and finally to 40 mg/day after 2 additional weeks with close monitoring for adverse events. The maximum dose was continued over 8 weeks, unless grade 3 or 4 adverse events attributable to pravastatin were observed. Immunosuppressive agents that were being taken at study entry were continued at the same dose. However, once the dose of these immunosuppressive agents was increased or other immunosuppressive agents were added, the patient was withdrawn from the study and considered a nonresponder.

The incidences and severity of adverse events attributable to pravastatin were evaluated according to the National Cancer Institute Common Toxicity Criteria, Version 2.0. To evaluate the efficacy of pravastatin, chronic GVHD was graded at study entry according to Akpek's prognostic model (*9*). Response was evaluated every 2 weeks for 12 weeks after the initiation of treatment as an intent-to-treat basis. Response in individual organs was defined as follows: A marked response was a change from Akpek's code 2 or 3 to code 1, a

good response was a change from code 3 to code 2, and no response was no change in code or progression. An overall response to treatment was defined as a marked or good response in at least one organ, without progression in any other organs. We planned to include 18 patients with target and lower response rates of 40% and 10% and alpha and beta errors of 5% and 10%, respectively.

The trough blood concentrations of cyclosporine or tacrolimus and the peak plasma concentration of pravastatin were measured every 2 weeks to evaluate interaction between pravastatin and these immunosuppressants. Immunologic changes were evaluated at weeks 2, 4, 8, and 12 by quantification of the CD4/CD8 ratio, the T-helper (Th)1/Th2 ratio, and the expression of human leukocyte antigen-DR on T cells, B cells, and monocytes. Immunologic data were compared between responders and nonresponders using a repeated measures analysis of variance after logarithmic transformation.

Eighteen patients with a median age of 44 years (range 20–68 years) were included in the study. There were 14 men and 4 women. The underlying disease was acute myeloblastic leukemia in seven, chronic myeloid leukemia in four, non-Hodgkin's lymphoma in three, acute lymphoblastic leukemia in two, myelodysplastic syndrome in one, and aplastic anemia in one. Thirteen and five patients received grafts from a related or an unrelated donor, respectively. Ten of them demonstrated chronic GVHD of progressive onset. All patients but one demonstrated extensive chronic GVHD before starting pravastatin, and nine patients were receiving prednisolone. The grade of chronic GVHD at study entry according to Akpek's prognostic model is shown in Table 1. Seven patients, 10 patients, and 1 patient were grouped into the low-, intermediate-, and high-risk groups, respectively.

Pravastatin was well tolerated, and no patients developed grade 3 or 4 adverse events attributable to pravastatin. Treatment was discontinued in three patients between 14 and 41 days after starting pravastatin because of unrelated causes, including painful oral chronic GVHD, infection, and interstitial pneumonitis. According to each organ, a response was observed in the skin score in two patients, mouth score in five patients, eye score in two patients, liver score in three patients, and platelet count score in one patient (Table 1). An overall response was seen in five patients (28%). Pravastatin did not act through the interaction with cyclosporine or tacrolimus, because an increase in these blood levels was not observed after the administration of pravastatin (data not shown). The serum pravastatin concentration on day 42 was not different between responders and nonresponders (median 157.5 ng/mL vs. 253.1 ng/mL, $P=0.53$). The serum total cholesterol level significantly decreased from 6.37 mmol/L (standard deviation [SD] 1.79) before treatment to 5.67 mmol/L (SD 1.40, $P=0.0095$) and 4.77 mmol/L (SD 1.99, $P=0.0001$) on days 14 and 84 after starting pravastatin, respectively. The initial cholesterol response (ratio between cholesterol level on day 14 and before treatment) was significantly better in GVHD responders (0.78 vs. 0.95, $P=0.029$).

The Th1/Th2 ratio before the administration of pravastatin was greater than 1.0 in all but one patient. The Th1/Th2 ratio at study entry tended to be lower in responders than in nonresponders and became even lower after pravastatin treatment in responders, but not in nonresponders, although these differences were not statistically significant (Fig. 1, $P=0.22$). The CD4/CD8 ratio and the expression of human leukocyte antigen-DR on T cells, B cells, and monocytes did not change after treatment (data not shown).

This study demonstrated that pravastatin at 40 mg/day can be safely administered in patients with refractory chronic GVHD, including those taking cyclosporine. The overall response of 28% was similar to that with other alternative salvage treatments including tacrolimus, mycophenolate mofetil, thalidomide, and so on (*3*). However, considering the safety profile of pravastatin, it may be worthwhile for patients with chronic GVHD, especially in those with a coexisting infection that precludes severely immunosuppressive treatments. We chose pravastatin among many statins because it is hydrophilic and was considered to be less likely to cause rhabdomyolysis than other lipophilic statins (*10, 11*). However, atorvastatin, lovastatin, and simvastatin have stronger in vitro immunosuppressive effects than pravastatin, and thus they may also have greater in vivo effects against chronic GVHD (*7, 8*).

There is some controversy whether human chronic GVHD is a Th1 or Th2 disease. The immunophenotypic analyses in this study clearly showed that Th1 cells were dominant in patients with chronic GVHD. The efficacy of statin against rheumatoid arthritis, a Th1 disease, has been demonstrated clinically (*12*). In a mouse model of chronic and relapsing

TABLE 1. Severity of chronic graft-versus-host disease in each organ and the response to pravastatin

	Severity code before treatment				Response to treatment				
Each organ	1	2	3	NE	Marked	Good	NC	PD	NE
Performance status	16	2	0	0	0	0	18	0	0
Skin and fascia	6	6	4	2	1	1	13	2	1
Mouth	5	11	2	0	3	2	12	1	0
Eye	7	8	3	0	2	0	13	2	1
Liver enzyme	4	5	9	0	2	1	13	2	0
Thrombocytopenia	14	1	3	0	0	1	15	2	0

Overall response	Responder	5 (28%)
	Nonresponder	13

NE, not evaluable; NC, no change; PD, progressive disease.

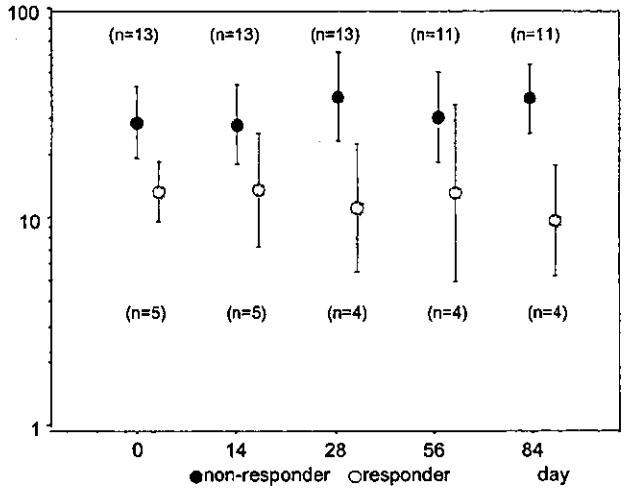

FIGURE 1. Serial changes in the T-helper (Th)1/Th2 ratio in responders and nonresponders. Data are shown as geometric mean and standard error.

experimental autoimmune encephalomyelitis, oral atorvastatin promoted a Th2 bias and reversed paralysis through the inhibition of STAT4 phosphorylation and the induction of STAT6 phosphorylation (*13*). Although we did not find a statistically significant association between the Th1/Th2 ratio and the response to pravastatin, pravastatin might have ameliorated chronic GVHD by inducing a Th2 shift.

In conclusion, our experience suggests that pravastatin may be safe and effective for the treatment of refractory chronic GVHD. However, a double-blind, randomized, controlled trial is needed to evaluate its true efficacy against refractory chronic GVHD.

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Allografting for older patients

Allogeneic myeloablative transplantation for patients aged 50 years and over

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Summary:

Allogeneic hematopoietic stem cell transplantation (HSCT) has been performed mainly for young patients due to concern about the high incidence of treatment-related mortality (TRM). Recent advances to reduce TRM by using peripheral blood stem cells or nonmyeloablative conditioning regimens have increased the age limit for this procedure, and correctly identifying the indication for transplant is essential for older patients. In this study, we analyzed data from 398 patients aged 50 or over selected from 5147 patients, who received conventional allogeneic HSCT (c-HSCT). Patients aged 50 or older showed inferior outcomes for TRM and overall survival (OS). Mulitivariate analyses confirmed that an age of 50 or over was an independent risk factor for TRM ($P<0.0001$) and OS ($P<0.0001$). Among patients aged 50 or older, increasing age remained an adverse factor for OS ($P=0.0213$). Regimens including total-body irradiation (TBI) correlated with a higher risk of TRM and a lower OS for older patients ($P=0.0095$ and 0.0303, respectively). These findings indicate that allogeneic c-HSCT should be offered to patients over 50 years only if the increased risk of TRM is acceptable, and that a non-TBI regimen is preferable when the transplant is performed.
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Allogeneic hematopoietic stem cell transplantation (HSCT) is now accepted as a curative therapy for patients with hematological malignancies and aplastic anemia. Despite its major potential as a cure, HSCT has been generally limited to younger patients due to concern about the high incidence of treatment-related mortality (TRM) for older patients. Recent advances have contributed to expanding its application. The use of peripheral blood (PB) cells instead of harvested bone marrow (BM) as a source of hematopoietic stem cells has been demonstrated to produce more rapid hematological recovery,[1,3] and has the advantage of a reduced risk of infectious complications during the early post transplant period. Moreover, development of nonmyeloablative transplantation (NST), a novel approach to reduce the intensity of conditioning regimens, has allowed older patients unsuitable for conventional HSCT (c-HSCT) to become transplant candidates.[4–6] These developments have made it necessary to decide on the appropriate option for older patients, especially those over 50 years, not only considering whether allogeneic HSCT should be performed, but also what type of transplant. The NST approach is now being investigated, but the effectiveness and safety of c-HSCT for such patients also needs evaluation. For this purpose, we analyzed data from 5147 patients who underwent allogeneic c-HSCT and whose transplants were reported to the Japan Society for Hematopoietic Cell Transplantation (JSHCT). A total of 398 patients over the age of 50 years were included in this study.

Patients and methods

Patients

The study population consisted of 5147 adult patients, 16 years of age or older, who were reported to the JSHCT as having undergone allogeneic c-HSCT for hematological disorders between January 1991 and December 2001. Patients who had received NST or cord blood transplantation were excluded, as were those who had undergone second or subsequent transplants. Information on surviving patients was updated annually. This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT.

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Statistical analysis

The primary end point of the analyses was to assess the influences of patient age on TRM and overall survival (OS). The secondary end point was to assess these effects on relapse, the development of grade II–IV acute graft-versus-host disease (GVHD) and extensive chronic GVHD. Whole-population analyses compared patients aged 50 years or older to those younger than 50. The analyses of the subgroup including only patients aged over 50 were performed using age as a continuous variable. OS was defined as time from the day of transplant to death or last follow-up. Relapse was defined as hematological recurrence, and only those with malignant diseases were evaluated. Patients never achieving complete remission (CR) after transplant were considered to have had a recurrence on day 0. TRM was defined as death while in continuous CR. Acute and chronic GVHD were evaluated according to standard criteria.[7,8] Those who died before engraftment were excluded from the analysis of acute GVHD, and those who died before day 100 were excluded from the analysis of chronic GVHD. The Cox proportional hazards regression model was used to evaluate the independent effect of age, sex, years of transplant, disease status, donor type, graft source, conditioning regimen and GVHD prophylaxis. Hazard ratio (HR) was calculated in conjunction with a 95% confidence interval (CI). For disease status, those with hematological malignancies in CR at the time of transplant, those in chronic phase of chronic myeloid leukemia (CML), those with refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS) of myelodysplastic syndrome (MDS), and all those with nonmalignant diseases were defined as being at standard risk, while those in other situations were defined as being at advanced risk. All donors except for human leukocyte antigen (HLA)-identical siblings were classified as alternative donors. For assessment of the effect of graft source, patients receiving both BM and PB stem cells (2% of the entire population) were excluded. Patients receiving GVHD prophylaxis other than cyclosporine (CSP)- or tacrolimus (FK506)-based regimens (3% of the entire population) were excluded from assessment of the effects of GVHD prophylaxis. Distributions of variables between the two age groups were compared by using the χ^2 test. Kaplan–Meier survival analyses were performed to estimate probability of OS, relapse and TRM. For relapse and TRM, each was used as a censored event for the other. Differences between groups were compared by means of the log-rank test. Stat View 5.0 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. The median age for the entire population was 34 years (range: 16–67). In all, 398 (8%) patients were 50 years of age or older (including 16 patients aged 60 years or older, and 77 patients aged 55–59 years), and 4749 (92%) patients were younger than 50 years. The two populations differed in the distributions of diagnosis, disease status, graft source, donor type and use of methotrexate (MTX). In the older group, the frequency of MDS and non-Hodgkin's lymphoma (NHL) was comparatively high, while that of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) was low. Older patients were more likely to be at advanced risk, to receive PB stem cells, to receive donations from HLA-identical siblings, and to receive no MTX. The distributions of sex, conditioning regimen (total-body irradiation [TBI] regimen *vs* non-TBI regimen) and GVHD prophylaxis (CSP- *vs* FK506-based regimen) were similar for the two groups. As shown in Figure 1, the proportion of older patients undergoing allogeneic c-HSCT has increased over time.

Transplant outcome

The median follow-up period for surviving patients was 1208 days. TRM was significantly higher for the older group than for the younger group (log-rank, $P<0.0001$; Figure 2a). The estimated probabilities were 16.5 *vs* 11.8% at 100 days, and 34.7 *vs* 22.7% at 1 year, respectively. The relapse rate was also higher for the older group than for the younger group, with respective probabilities of 36.4% and 29.7% at 4 years (log-rank, $P=0.0042$; Figure 2b). Consequently, age at transplant strongly correlated with survival (log-rank, $P<0.0001$; Figure 2c). The probability of OS at 4 years was 35.6% for patients 50 years or older, and 53.3% for those under 50 years.

Risk factor analysis

Nine pretransplant parameters were selected to be included for the subsequent multivariate analyses, ie, age group, sex, year of transplant, disease status, donor type, graft source, conditioning regimen and GVHD prophylaxis (CSP- *vs* FK506-based regimen and MTX *vs* no MTX). When the risk of GVHD was analyzed as times to events, patients aged 50 or older tended to be at greater risk of developing grade II–IV acute GVHD and extensive chronic GVHD ($P=0.0886$ and 0.0844, respectively). The results of multivariate analyses for outcome are shown in Table 2. Although the age group had no effect on risk of relapse ($P=0.3165$), being 50 or older was associated with an increased risk of TRM ($P<0.0001$), and had a significantly negative impact on OS ($P<0.0001$). In addition to patient age, transplants performed in the early years, advanced risk disease, donation from alternative donors and omission of MTX were identified as adverse factors for both TRM and OS. Male sex and a CSP-based regimen also correlated with an increased risk of TRM. The risk of relapse was significantly higher among those with advanced risk disease and among those receiving transplants from HLA-identical siblings.

Analysis of the subgroup consisting of patients aged 50 or older

To identify the risk factors and their effect on transplant outcome in older patients, subgroup analyses were

Table 1 Patient characteristics

	Total $n=5147$	*Age* $\geqslant 50$ $n=398$ (8)	*Age* <50 $n=4749$ (92)	*P-value*
Age				
Median	34	52	32	
Range	16–67	50–67	16–49	
Sex				$P=0.4761$
Male	3160 (61)	251 (63)	2909 (61)	
Female	1987 (39)	147 (37)	1840 (39)	
Disease				$P<0.0001$
AML	1427 (28)	91 (23)	1336 (28)	
ALL	1054 (20)	58 (14)	996 (21)	
CML	1334 (26)	94 (24)	1240 (26)	
MDS	539 (10)	82 (21)	457 (10)	
NHL	351 (7)	45 (11)	306 (6)	
AA	297 (6)	10 (3)	287 (6)	
Others	145 (3)	18 (4)	127 (3)	
Disease status				$P<0.0001$
Standard risk	3638 (71)	220 (55)	3418 (72)	
Advanced risk	1509 (29)	178 (45)	1331 (28)	
Graft source				$P<0.0001$[a]
BM	4569 (89)	292 (74)	4277 (90)	
PB	484 (9)	83 (21)	401 (9)	
BM + PB	81 (2)	18 (5)	63 (1)	
Donor type				$P<0.0001$
HLA-identical sibling	3419 (67)	316 (80)	3103 (66)	
Alternative donor	1704 (33)	78 (20)	1626 (34)	
TBI regimen				$P=0.6820$
Y	3385 (66)	245 (62)	3140 (66)	
N	1753 (34)	152 (38)	1601 (34)	
GVHD prophylaxis				$P=0.8692$[b]
CSP-based regimen	4549 (89)	343 (87)	4206 (89)	
FK506-based regimen	437 (8)	32 (8)	405 (9)	
Others	135 (3)	21 (5)	114 (2)	
MTX				P = 0.0049
Y	4746 (93)	353 (89)	4393 (93)	
N	375 (7)	43 (11)	332 (7)	
Year of transplant[c]				$P<0.0001$
1991–1996	2505 (49)	72 (18)	2433 (51)	
1997–2001	2642 (51)	326 (82)	2316 (49)	

AML = acute myeloid leukemia; ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia; NHL = non-Hodgkin's lymphoma; AA = aplastic anemia; BM = bone marrow; PB = peripheral blood; HLA = human leukocyte antigen; TBI = total-body irradiation; GVHD = graft-versus-host disease; CSP = cyclosporine; FK506 = tacrolimus; MTX = methotrexate.
Values in parentheses are percentages.
[a]Compared between BM and PB. [b]Compared between CSP and FK506. [c]Details are shown in Figure 1.

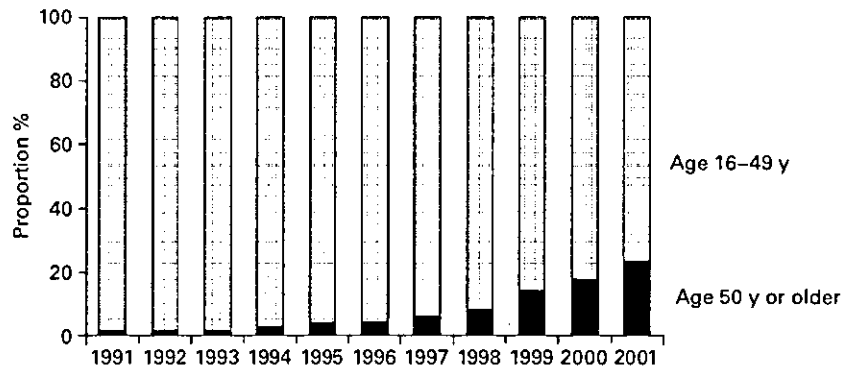

Figure 1 The proportion of patients aged 50 or older undergoing allogeneic myeloablative transplantation. The proportion of patients aged 50 or older among those who received allogeneic conventional transplantation has increased over time between 1991 and 2001.

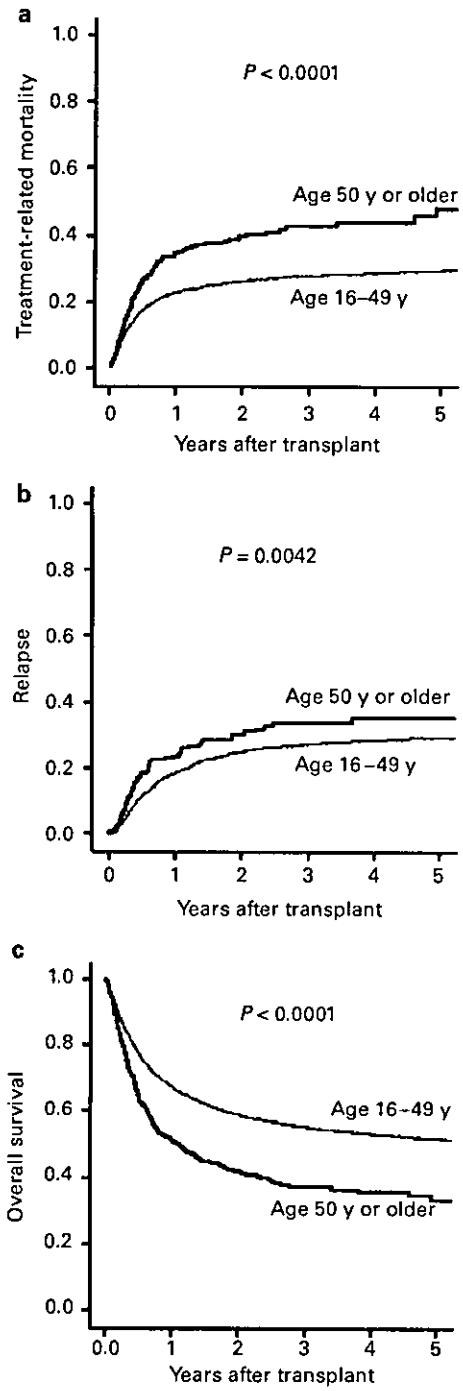

Figure 2 Treatment-related mortality, relapse and overall survival by age group. (a) The probability of treatment-related mortality was significantly higher for patients 50 years old or older (log-rank, $P<0.0001$). (b) Relapse rate was also higher for the older group (log-rank, $P=0.0042$), but the difference was not significant in multivariate analysis ($P=0.3165$). (c) Age at transplant was strongly associated with overall survival (log-rank, $P<0.0001$).

performed for the 398 patients aged 50 or over (Table 3). As described in *Patients and methods*, age at transplant was included in the Cox proportional hazards regression model as a continuous variable. Multivariate analysis demonstrated that increased age remained as an influence on OS for this older population ($P=0.0213$). Advanced risk disease increased the risk of relapse ($P<0.0001$), and correlated with inferior OS ($P<0.0001$). Transplants from alternative donors, omission of MTX and TBI-containing regimen were also independent risks for TRM ($P=0.0429$, 0.0175 and 0.0095, respectively). Kaplan–Meier curves based on the type of conditioning regimen are shown in Figure 3. TBI-containing regimens were associated with a higher probability of TRM without improvement of relapse rate, thus resulting in a significantly lower probability of OS (log-rank, $P=0.0256$ for TRM, $P=0.6448$ for relapse, and $P=0.0255$ for OS). The influence on TRM and OS was of statistical significance in multivariate analysis ($P=0.0095$ for TRM and $P=0.0303$ for OS).

Discussion

Allogeneic HSCT has been preferentially used for younger patients primarily due to concern about the high incidence of treatment-related morbidity and mortality. Generally, advanced age is considered to be a poor prognostic factor following HSCT. The major reasons for this are (1) underlying medical complications or organ dysfunction, (2) delay in drug metabolism potentiating the toxicity of conditioning regimens, and (3) a high incidence of GVHD as reported previously.[9,12] Although the upper age limit for allogeneic HSCT has been set at around 50 years in many institutions, recent advances in HSCT have raised this limit. The use of PB instead of BM as a stem cell source has been demonstrated to reduce the duration of neutropenia,[1,3] which is especially beneficial for older patients exposed to a greater risk of life-threatening infection. Another major advance is the development of NST. The reduced toxicity of conditioning regimens has made it possible for this procedure to be used for older patients ineligible for c-HSCT. Published studies of NST have reported its use for patients in their 60s and even 70s.[4–6] Changes in recent years have expanded the range of treatment options, thus making assessment of c-HSCT for older patients an urgent necessity. In this study, we evaluated 398 older patients who underwent allogeneic c-HSCT and compared their outcomes with those for 4749 patients less than 50 years old. To our knowledge, this is the largest analytical study performed to date of allogeneic HSCT for patients aged 50 or older.

This study demonstrated that age 50 or more was strongly associated with a higher risk of TRM and inferior OS. Univariate analysis showed the effect of age on the incidence of relapse to be statistically significant, but multivariate analysis did not. This is attributable to the fact that advanced risk disease and transplantation from HLA-identical siblings, both of which were independent adverse factors for relapse, were more common among older patients. A study by the International Bone Marrow Transplant Registry (IBMTR) analyzed a total of 2180 leukemia patients, including 80 patients aged 50 or older, who had received allogeneic HSCT from HLA-identical siblings.[13] Patients were divided into four age groups and

Table 2 Hazard ratios and 95% confidence intervals of risk factors with statistical significance in multivariate analysis

	HR (95% CI)	*P-value*	*Adverse factor*
Treatment-related mortality			
Donor type	2.38 (2.10–2.69)	$P<0.0001$	Alternative donor
Disease status	1.97 (1.74–2.23)	$P<0.0001$	Advanced risk
Age	1.70 (1.38–2.09)	$P<0.0001$	Age $\geqslant 50$
Year of transplant	1.05 (1.02–1.07)[a]	$P=0.0003$	Early years
MTX	1.48 (1.18–1.86)	$P=0.0003$	No MTX
Sex	1.15 (1.02–1.30)	$P=0.0216$	Male
GVHD prophylaxis	1.26 (1.03–1.55)	$P=0.0286$	CSP-based regimen
Relapse			
Disease status	3.82 (3.35–4.34)	$P<0.0001$	Advanced risk
Donor type	1.18 (1.01–1.38)	$P=0.0341$	HLA-identical sibling
Overall survival			
Disease status	2.72 (2.49–2.97)	$P<0.0001$	Advanced risk
Donor type	1.59 (1.45–1.75)	$P<0.0001$	Alternative donor
Age	1.51 (1.30–1.77)	$P<0.0001$	Age $\geqslant 50$
Year of transplant	1.04 (1.02–1.06)[a]	$P<0.0001$	Early years
MTX	1.24 (1.04–1.47)	$P=0.0161$	No MTX

HR = hazard ratio; 95% CI = 95% confidence interval; MTX = methotrexate; GVHD = graft-versus-host disease; CSP = cyclosporine.
HR corresponds to risk of death or relapse.
[a]Modeled as a continuous variable; hazard ratio is shown per year.

Table 3 Hazard ratios and 95% confidence intervals of risk factors for patients aged 50 or older with statistical significance in multivariate analysis

	HR (95% CI)	*P-value*	*Adverse factor*
Treatment-related mortality			
Conditioning regimen	1.80 (1.15–2.80)	$P=0.0095$	TBI
MTX	2.23 (1.15–4.31)	$P=0.0175$	No MTX
Donor type	1.62 (1.02–2.60)	$P=0.0429$	Alternative donor
Relapse			
Disease status	2.53 (1.59–4.03)	$P<0.0001$	Advanced risk
Overall survival			
Disease status	1.84 (1.38–2.46)	$P<0.0001$	Advanced risk
Age	1.06 (1.01–1.11)[a]	$P=0.0213$	Older age
Conditioning regimen	1.41 (1.03–1.93)	$P=0.0303$	TBI

HR = hazard ratio; 95% CI = 95% confidence interval; TBI = total-body irradiation; MTX = methotrexate; HR corresponds to risk of death or relapse.
[a]Modeled as a continuous variable; hazard ratio is shown per year.

those with advanced leukemia and aged 45 or older showed a slightly higher risk of TRM, but no difference in leukemia-free survival was observed among age groups. Another study of a large series was that reported by the European Group for Bone Marrow Transplantation (EBMT), which compared 192 patients over 40 with 1119 younger patients.[14] Only those with AML and aged 45 or older were at higher risk of TRM but without any difference in terms of OS. These data as well as ours should be interpreted with caution because patients actually undergoing allogeneic HSCT are thought to be highly selected. However, in our study covering the largest number of patients to date, it should be emphasized that even highly selected patients showed poorer prognosis. It is in accordance with previously reported findings that the incidence of GVHD was higher in older patients,[9,12] which almost reached statistical significance in our study. The higher incidence of GVHD has been proposed as one of the causes contributing to the higher risk of TRM.

Among patients aged over 50 years, increasing age remained an adverse factor for OS. As expected, advanced risk disease was associated with an inferior outcome in terms of relapse and OS. Notably, patients receiving a TBI-containing regimen were at higher risk of TRM and demonstrated a lower probability of OS. Although radiation is effective as part of the conditioning regimen, no conclusion has been reached as to whether a TBI-containing regimen is superior or inferior to a non-TBI regimen.[15,16] Two of five randomized controlled trials showed the superiority of TBI-regimens for survival.[17,18] but no difference was observed in the other three trials.[19–21] It should be remembered that previous trials have not included older patients according to each eligibility criterion. In the study reported here, TBI-regimens were

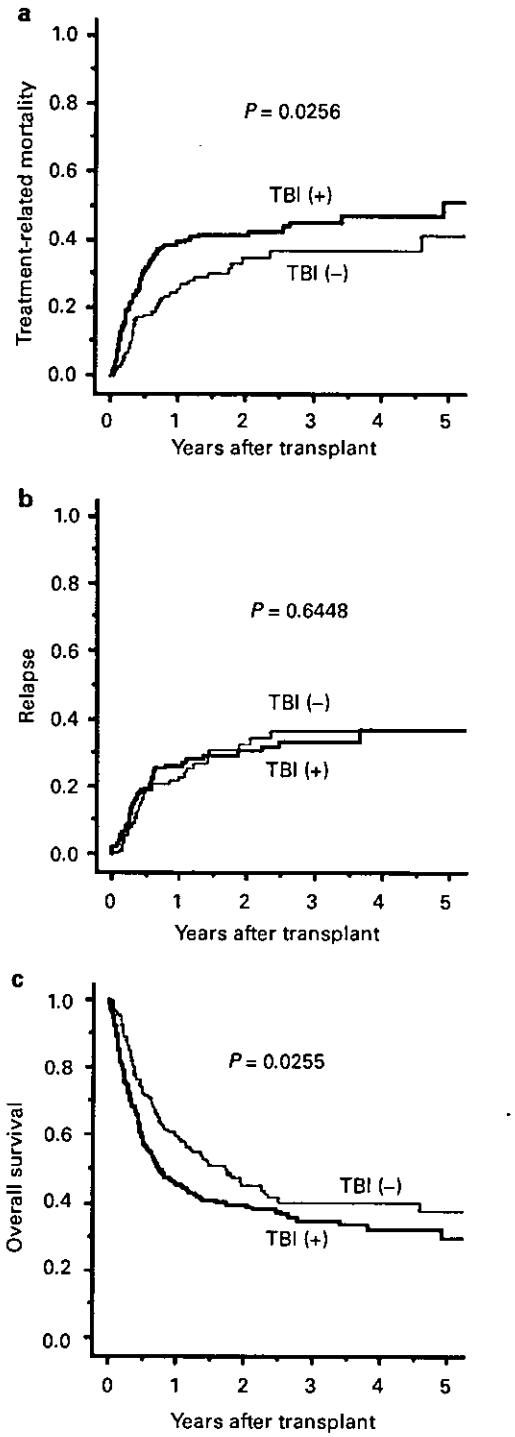

Figure 3 Treatment-related mortality, relapse and overall survival by conditioning regimen for patients aged 50 or older. TBI-containing regimens were associated with (a) higher incidence of TRM, (b) with no improvement of relapse rate (log-rank, $P = 0.0256$ and 0.6448, respectively). (c) Patients aged 50 or older receiving a TBI-containing regimen showed poorer survival (log-rank, $P = 0.0255$).

found to be more toxic and to worsen OS for patients over 50 years, while the detrimental effect of TBI was not demonstrated among younger patients.

In summary, patient age at transplantation is a strong indicator for outcome after allogeneic c-HSCT. This treatment for patients aged 50 or older is associated with a higher risk of TRM and with a lower OS than for those under 50 years. Furthermore, conditioning regimens including TBI enhance negative effects. It is therefore suggested that allogeneic c-HSCT should be offered to patients 50 years of age or older only if the higher risk of TRM is acceptable. When the transplant is performed, a non-TBI regimen is preferable. Prospective comparative analyses of c-HSCT and NST are also needed for this population group.

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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

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Abstract

Imatinib mesylate is a relatively new drug that targets the BCR-ABL chimeric protein, the molecular basis of chronic myeloid leukemia (CML). A phase II clinical trial in 39 Japanese patients in the first chronic phase of CML was conducted with imatinib mesylate at a dose of 400 mg/day. Hematologic complete response was obtained in 92.3% of the patients, complete cytogenetic response (CR) was obtained in 43.6%, and major partial CR was obtained in 20.5% of the patients. Although 29 of 39 patients required an adjustment of dosing because of grade 3 or 4 adverse events, most of the events were reversible, and 25 of the 29 patients were able to resume therapy. Between day 15 and day 35, grade 3 or 4 neutropenia and/or leukocytopenia occurred in 13 patients, and grade 3 thrombocytopenia occurred in 5 patients. Overall, nonhematologic grade 3 adverse events occurred in 28.2% of the patients. These data support the use of imatinib mesylate as the treatment of choice for chronic-phase CML patients.
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Key words: Imatinib mesylate; Chronic myeloid leukemia; Phase II clinical study

1. Introduction

Chronic myeloid leukemia (CML) is a hematologic disease arising from the malignant transformation of hematopoietic stem cells and is characterized by the excessive proliferation of myeloid cells. The characteristic feature of CML is the presence of the Philadelphia chromosome (Ph) in hematopoietic cells, which is caused by the reciprocal translocation of t(9;22) [1-3]. The fused transcripts of the *abl* and *bcr* genes produce the chimeric BCR-ABL protein, a constitutively active tyrosine kinase [4].

The clinical course of CML consists of 3 phases. The chronic phase is a period of proliferation and differentiation of myeloid cells. After several years, the chronic phase proceeds to the accelerated phase and terminates in blast crisis.

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which is characterized by the transformation of the hematopoietic cells to acute blast cells with a clinical picture similar to that of acute leukemias.

Considerable progress has been made in the therapy of CML over the past 20 years. Interferon α (IFN-α) has been shown to reduce or diminish Ph^+ malignant cells and prolong the duration of the chronic phase [5,6]. Allogeneic hematopoietic stem cell transplantation (HSCT) has been established as the only mode of curative therapy, especially for patients in the chronic phase of CML, and more than two thirds of chronic-phase patients who receive an HSCT from an HLA-matched sibling or unrelated donor can be considered cured of the disease [7,8].

In the 1990s, a promising new drug, imatinib mesylate (Gleevec; Novartis Pharmaceuticals, East Hanover, NJ, USA), targeting the BCR-ABL chimeric protein [9,10] was developed. This potent competitive inhibitor of BCR-ABL tyrosine kinase activity specifically blocks proliferation and promotes the in vitro apoptosis of cells containing this protein.

Two clinical phase I studies were first conducted. One was carried out for patients with chronic-phase CML [11], and the other was for patients with blast crisis CML and acute lymphoblastic leukemia [12]. These studies were followed by a large-scale international phase II study for IFN-resistant patients with chronic-phase CML [13]. A clinical phase I study for Japanese patients was also conducted for IFN-resistant patients with chronic-phase CML (unpublished data).

The analysis of these clinical trials clearly demonstrated that (1) the drug-related adverse events of imatinib mesylate were minimal and clinically acceptable and (2) the response to imatinib mesylate was remarkable. Kantarjian et al reported that 60% of IFN-resistant patients achieved a major cytogenetic response (CR) and that 95% showed a complete hematologic response (HR) [13].

In the present study, a phase II clinical trial of imatinib mesylate was conducted at a dose of 400 mg/day in Japanese patients with chronic-phase CML. Primary end points were HR and the safety of imatinib mesylate, and secondary end points were the duration of HR and the rate of CR.

2. Patients and Methods

2.1. Patient Eligibility Criteria

Patients with chronic-phase CML fulfilling the following criteria were enrolled in this phase II study. Patient age ranged from 15 years to 74 years. Patients were classified into 1 of the following 4 treatment categories: (1) no HR, consisting of patients who failed to achieve complete HR with the administration of IFN for more than 3 months or who failed to maintain a complete HR by IFN therapy; (2) no CR, consisting of patients who showed more than 65% Ph^+ cells in the bone marrow with the administration of IFN for more than 1 year or who showed a 1-month increase of Ph^+ cells of greater than 30% in the bone marrow despite IFN administration; (3) IFN-intolerant patients who stopped receiving IFN because of adverse events caused by IFN; and (4) patients with no history of IFN therapy.

Exclusion criteria included the following characteristics: (1) fewer than 10,000/μL white blood cells in the peripheral blood; (2) a serum bilirubin or creatinine level elevated more than 2-fold higher than the upper limit of the normal value; (3) a serum glutamic-oxaloacetic transaminase or glutamic-pyruvic transaminase level elevated more than 3-fold higher than the upper limit of the normal value; (4) eligibility for allogeneic HSCT from an HLA-identical sibling or unrelated donor; (5) a history of HSCT; (6) cardiac disease of more than grade 3 according to the New York Heart Association scale; and (7) a history of administration of busulfan within the previous 6 weeks, IFN-α within 2 weeks, cytosine arabinoside within 2 weeks, or hydroxyurea within 1 week.

Each patient signed an informed consent form at the time of study entry. The study was approved by the institutional review board of each institution.

2.2. Study Design and Treatment Schedule

The study was designed by the investigators and representatives of the sponsor, Novartis Pharmaceuticals. The data were collected and analyzed with the use of the data-management and statistical support of Novartis Pharmaceuticals in close collaboration with the investigators.

A 400-mg dose of imatinib mesylate was administered orally once a day 2 hours after breakfast for 12 weeks. Optional continuation of imatinib mesylate administration was scheduled thereafter.

When an adverse event occurred, the dosage was adjusted according to the following rules. When a grade 3 or 4 leukocyte (neutrophil) or platelet adverse event was observed, imatinib mesylate was discontinued until cell counts recovered to grade 2 or less. When a grade 2 or greater nonhematologic adverse event was observed, imatinib mesylate was discontinued until the patient improved to grade 1, and then it was readministered at the same dose in cases of a grade 2 event and at a 100-mg reduced dose in cases of a grade 3 event.

2.3. Assessment of Tumor Response and Toxicity

Tumor response was determined according to the following response criteria. A complete HR was defined as reduction of the leukocyte count to less than 10,000/μL and the platelet count to less than 500,000/μL for at least 4 weeks. CR was determined by the percentage of Ph^+ cells in the bone marrow. From the chromosomal analysis of 20 cells in metaphase, CR was further categorized as complete CR (no Ph^+ cells), major partial CR (1%-35% Ph^+ cells), minor partial CR (36%-65% Ph^+ cells), minimal partial response (66%-95% Ph^+ cells), and no response (96%-100% Ph^+ cells). When chromosomal analysis was not available, the results of fluorescent in situ hybridization analysis were substituted, and finding less than 1.3% Ph^+ cells in a fluorescent in situ hybridization analysis was regarded as a complete CR.

Adverse events were graded according to National Cancer Institute Common Toxicity Criteria version 2.0, and the cumulative tumor response rate was calculated by the Kaplan-Meier method.

Table 1.
Patient Characteristics*

	Prior Interferon Therapy Status				
	No Hematologic Response (n = 7)	No Cytogenetic Response (n = 15)	Interferon Intolerant (n = 10)	No Interferon Therapy (n = 7)	Total (N = 39)
Age, y	52 (32-71)	56 (26-66)	58 (23-69)	55 (35-71)	56 (23-71)
Body weight, kg	65.0 (45.0-77.0)	61.0 (49.0-86.0)	61.5 (42.7-79.0)	63.5 (44.3-75.3)	62.0 (42.7-86.0)
Male/female sex, n	4/3	10/5	6/4	4/3	24/15
Performance status (grade 0/1), n	7/0	13/2	10/0	7/0	37/2
Duration from diagnosis, n					
≤6 mo			1	2	3
6-12 mo	2		2	3	7
12-24 mo		6	2		8
24-60 mo	2	5	1	1	9
>60 mo	3	4	4	1	12
Duration of interferon therapy, n					
No therapy				7	7
≤6 mo			4		4
6-12 mo	2		1		3
12-24 mo	1	8	2		11
24-60 mo	2	5	2		9
>60 mo	2	2	1		5
WBC count before imatinib treatment, $\times 10^4/\mu L$	2.44 (1.447-5.37)	1.30 (0.94-3.15)	2.21 (1.045-9.1)	1.43 (1.2-9.42)	1.45 (0.94-9.42)
Platelet count before imatinib treatment, $\times 10^4/\mu L$	35.2 (14.1-168.3)	27.8 (8.1-143.0)	30.7 (17.6-67.8)	30.25 (27.7-66.6)	30.35 (8.1-168.3)
Splenomegaly (>10 cm below the costal margin)	0	0	0	0	0

*Data are presented as the median (range) where appropriate. WBC indicates white blood cell.

3. Results

3.1. Patients and Treatment

Forty-three patients were enrolled between January 22, 2001, and May 10, 2001. Four patients were excluded because of low white blood cell counts (fewer than 10,000/μL just before scheduled administration) according to the protocol, so that 39 patients were evaluable for safety and response. The characteristics of these patients are summarized in Table 1. Twenty-two patients had a history of no response to prior IFN therapy, 10 were intolerant to prior IFN treatment, and 7 had undergone no prior IFN therapy at the time of registration. Patient ages ranged from 23 to 71 years (median, 56 years). Twenty-four patients were male, and 15 were female.

The duration of imatinib mesylate administration ranged from 11 to 292 days (median, 237 days). The dosage was adjusted for 32 patients because of adverse effects (30 patients), no tumor effect (1 patient), or both (1 patient). Of these 32 patients, drug administration was stopped in 29 patients, transiently in 27 patients and permanently in 2 patients. The dosage of imatinib mesylate was increased in 2 patients because of insufficient tumor response.

Bone marrow aspiration was performed every 3 months for the assessment of CRs, and clinical responses, including any adverse events of imatinib mesylate, were observed for a median of 270 days (range, 15-300 days) after the initial administration.

3.2. Response Rate

Complete HR was obtained in 36 (92.3%) of 39 patients and required 6 to 56 days (median, 15 days) of treatment to achieve (Figure 1; Table 2). Two patients who did not obtain HR or CR showed disease progression to blast crisis on day 168 and day 248 after the administration of 400 mg/day. One patient received a subtherapeutic dose of imatinib mesylate because of the adverse effect of thrombocytopenia and showed no HR.

CR was assessed every 3 months, and the cumulative duration to achieve complete CR and major partial CR is

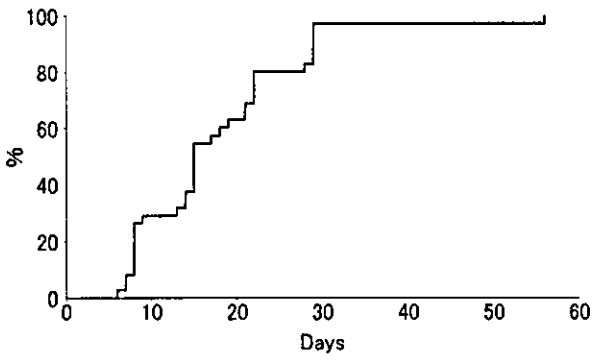

Figure 1. Cumulative days to achieve complete hematologic response (HR) after the administration of imatinib mesylate in patients with HR.

Table 2.
Response Rate of Imatinib Mesylate in Patients with Chronic-Phase Chronic Myeloid Leukemia

	Prior Interferon Therapy Status				
	No Hematologic Response (n = 7), n	No Cytogenetic Response (n = 15), n	Interferon Intolerant (n = 10), n	No Interferon Therapy (n = 7), n	Total (N = 39), n (%)
Hematologic response					
Complete remission	7	13	9	7	36 (92.3)
Not evaluable	0	2	1	0	3
Cytogenetic response					
Complete	3	8	3	3	17 (43.6)
Major partial	2	2	2	2	8 (20.5)
Minor partial	0	1	0	0	1 (2.6)
Minimal partial	0	0	2	1	3 (7.7)
No response	2	3	2	1	8 (20.5)
Not evaluable	0	1	1	0	2 (5.1)

shown in Figure 2. By 3 months after the start of therapy, 21% and 46% of patients achieved complete CR and major partial CR, respectively. The cumulative incidence of complete CR increased to 41% at 6 months and reached 44% during the clinical study period. There were no differences in CR rate according to prior IFN treatment status between the 4 groups.

3.3. Adverse Events

Hematologic adverse events were observed in 23 of 39 patients (Table 3). Neutropenia of grade 3 or 4 occurred in 14 patients, and thrombocytopenia of grade 3 or 4 occurred in 12 patients. Neutropenia appeared within 22 to 103 days (median, 46 days) after the administration of imatinib mesylate, and thrombocytopenia appeared within 15 to 197 days (median, 35 days).

Nonhematologic adverse events were observed in 36 patients (Table 3). Grade 3 and grade 1 to 2 skin rash occurred in 7 patients (17.9%) and 11 patients (28.2%), respectively. Grade 3 vomiting occurred in 2 patients (5.1%), and grade 3 myalgia occurred in 1 patient (2.6%). Other frequent grade 1 to 2 adverse events were nausea (43.6%), diarrhea (20.5%), fatigue (20.5%), edema of the eyelid (33.3%) or face (15.4%), muscle cramps (15.4%), taste disturbance (12.8%), and arthralgia (10.3%).

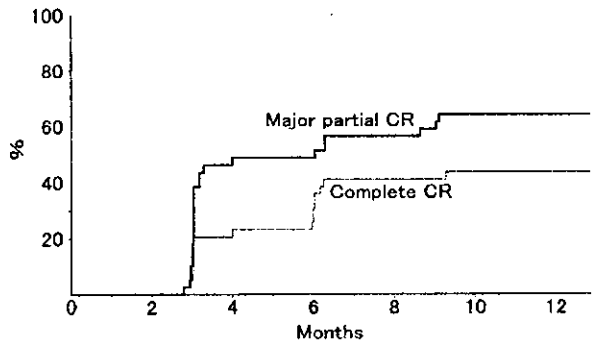

Figure 2. Cumulative incidence of major partial cytogenetic response (CR).

Liver dysfunction was observed in 2 patients (grade 2 and grade 3), and elevation of the serum alkaline phosphatase level (greater than grade 3) with no clinical signs and symptoms was noted in 10 patients.

Serious drug-related adverse events were reported in 9 patients. Neutropenia occurred in 6 patients, thrombocytopenia in 1, neutropenia and thrombocytopenia in 1, and herpes zoster in 1.

Only 2 patients permanently discontinued imatinib mesylate treatment, 1 patient at day 12 because of myalgia and the other because of recurrent grade 3 neutropenia. Of the other 29 patients who required dose adjustment or interruption, all patients resumed therapy again at 400 mg/day or with a decreased dose after they recovered from the adverse events.

3.4. Patient Survival

During the observation period, only 1 of the 39 patients died from progression of the disease into blast crisis.

4. Discussion

Imatinib mesylate has demonstrated excellent antileukemia effects in this Japanese phase II study of CML patients in the chronic phase, most of whom were IFN resistant or intolerant.

This phase II study was designed as part of a phase I/II study of imatinib mesylate for treating chronic-phase CML, and the dose was determined according to the data of the preceding dose-finding phase I study conducted with doses of 200, 400, and 600 mg/day of imatinib mesylate. The data from the phase I part of the study indicated that 600 mg/day was tolerable; however, 200 mg/day was not sufficient for the achievement of a CR (unpublished data). Thus, the phase II study was conducted at an imatinib mesylate dose of 400 mg/day for 8 weeks.

Complete HR was obtained in 92.3% of the patients, complete CR was obtained in 43.6%, and minor partial CR was obtained in 20.5% (Table 2). These response rates were comparable with those of a large-scale international phase II study using the same single dose of 400 mg/day in IFN-resistant patients in the chronic phase [13].

Table 3.
Adverse Events Related to Treatment with Imatinib Mesylate

Event	No. of Patients with Event, n (%)		
	Grades 1-4	Grade 3	Grade 4
Nonhematologic	36 (92.3)	11 (28.2)	0
Skin and subcutaneous tissue disorders			
Dermatitis	18 (46.2)	7 (17.9)	0
Eyelid edema	13 (33.3)	0	0
Face edema	6 (15.4)	0	0
Gastrointestinal disorders			
Nausea	17 (43.6)	0	0
Diarrhea	8 (20.5)	0	0
Vomiting	6 (15.4)	2 (5.1)	0
Abdominal pain	4 (10.3)	0	0
General disorders			
Fatigue	8 (20.5)	0	0
Edema	6 (15.4)	0	0
Muscle-skeletal, connective tissue, and bone disorders			
Muscle cramps	6 (15.4)	0	0
Arthralgia	4 (10.3)	0	0
Myalgia	4 (10.3)	1 (2.6)	0
Nervous system disorders			
Taste disturbance	5 (12.8)	0	0
Hematologic	24 (61.5)	21 (53.8)	7 (17.9)
Thrombocytopenia	15 (38.5)	11 (28.2)	1 (2.6)
Neutropenia	14 (35.9)	8 (20.5)	6 (15.4)
Leukopenia	13 (33.3)	10 (25.6)	2 (5.1)
Anemia	6 (15.4)	2 (5.1)	1 (2.6)

Although the patient body surface area (median, 1.68 m^2; range, 1.27-2.05 m^2) in this Japanese study cohort was smaller than that reported for non-Japanese studies, the frequency and grade of adverse events were similar. The serum concentrations of imatinib mesylate determined in the pharmacologic study of the phase I part of this Japanese study were not significantly different from those of an international phase I study. These results indicate that a dose of 400 mg/day is an appropriate initial dose of imatinib mesylate in adult CML patients in the chronic phase.

We consider it important to understand the time until response and the duration of response to imatinib mesylate, because alternative treatments, such as allogeneic HSCT from HLA-identical related or unrelated donors, could be one of the choices for imatinib mesylate–resistant patients. Our data indicate that administration for at least 6 months is necessary to achieve complete CR or major partial CR. When a patient is a poor responder (that is, he or she shows no response or minor partial CR after 3 or 6 months of administration), an increase of the dosage to more than 600 mg may be another choice for treatment. Kantarjian et al recently reported the efficacy and safety of 800 mg/day in treating CML in the chronic phase [14].

To predict the efficacy of imatinib mesylate for individual patients, Kaneta et al analyzed the expression profiles of CML cells from the 18 CML patients who were treated in this phase II study [15]. The analysis of complementary DNA microarrays representing 23,040 genes identified 79 genes that were expressed differentially between responders and nonresponders to imatinib mesylate. Validation of these findings is ongoing in another cohort of patients. Thus, these results may provide the first evidence that gene expression profiling can be used to predict the sensitivity of CML cells to imatinib mesylate.

Although 29 of the 39 patients had their imatinib mesylate dosing interrupted according to the dose-adjustment rules in the protocol because of adverse events, most of the events were reversible, and 25 patients resumed treatment. Between days 15 and 35, neutropenia and/or leukopenia of grade 3 or 4 occurred in 13 patients, and grade 3 thrombocytopenia occurred in 5 patients. We suspect that during this period, Ph^+ hematopoietic cells decreased or disappeared in the bone marrow in responsive patients and that normal hematopoiesis had not yet fully recovered. Therefore, close attention should be paid during this period to monitoring for the occurrence of these specific adverse hematologic events. The administration of granulocyte colony-stimulating factor might also be considered when severe neutropenia and neutropenic fever become evident [16].

Recently, a large-scale international randomized phase III clinical study in patients with newly diagnosed chronic-phase CML that compared imatinib mesylate with the combination of IFN and cytosine arabinoside clearly indicated that imatinib mesylate was more effective as first-line therapy for patients with chronic-phase CML [17]. Furthermore, Kantarjian et al reported a follow-up analysis of the phase II study in treating chronic-phase CML that showed that imatinib mesylate maintained favorable outcomes in chronic-phase CML after a median duration of therapy of 29 months [18]. However, because of reports that patients who did not achieve a CR with imatinib mesylate had significantly worse survival times than responding patients, we can recommend

that when such patients are identified, they should be immediately considered for alternative treatments [19].

Thus, the results of this study provide additional and corroborative evidence for using imatinib mesylate as the treatment of choice for chronic-phase CML patients.

Follow-up for more than 5 years will be required to evaluate the long-term safety and tolerance of this drug, the durability of responses, and the potential for cure, as well as to identify the situations in which alternative therapy can be recommended.

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Immature Granulocyte Fraction in the Peripheral Blood Is a Practical Indicator for Mobilization of CD34$^+$ Cells

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We propose a simple parameter that improves prediction of the number of CD34$^+$ cells in blood cells collected by apheresis for autologous peripheral blood stem cell (PBSC) transplantation following administration of granulocyte colony-stimulating factor. The percentage of immature granulocytes including myeloblasts, promyelocytes, myelocytes, and metamyelocytes (LSI for left-shift index) immediately prior to the start of each apheresis correlated with the number of CD34$^+$ cells in PBSC collections ($r = 0.79$, $P < 0.0001$, $Y = 0.227X - 0.99$, $R^2 = 0.623$) much better than did the white blood cell count ($r = 0.07$), currently the most commonly used predictor in deciding the initiation of apheresis. We then used receiver operating characteristic (ROC) curves to determine a cutoff point for LSI to prevent unnecessary apheresis. At LSI > 7.5, sensitivity and specificity of cutoff points in the probability of obtaining >1.0 × 10^6 CD34$^+$ cells/kg BW were 93.3% and 94.3% (95% CI, 91.4–100.0%), respectively. When LSI reaches 15.25, nearly 100% of apheresis will attain the target CD34$^+$ cell dose. These findings indicate that LSI is a useful and simple method for predicting the yield of CD34$^+$ cells before the start of PBSC collection and avoiding unnecessary apheresis. Am. J. Hematol. 77:223–228, 2004. © 2004 Wiley-Liss, Inc.

Key words: peripheral blood stem cell transplantation; harvest; CD34$^+$ cells

INTRODUCTION

Autologous peripheral blood stem cell (PBSC) transplantation following high-dose chemotherapy is considered an effective curative strategy for a wide variety of malignancies resistant to conventional treatment or with poor prognostic factors [1–3]. Compared with bone marrow cells, PBSCs offer ease of collection and rapid rates of engraftment [4]. However, the timing of mobilization and yield are highly variable and thus the prediction of the number of mobilized stem cells in the peripheral blood (PB) is difficult. Such information is essential for deciding the optimal time for initiation of apheresis. A strong positive correlation is seen between the numbers of circulating CD34$^+$ cells and colony-forming unit granulocyte-macrophage (CFU-GM) [2,5,6], and it is now believed that the number of circulating CD34$^+$ cells is likely to represent the efficacy of stem cell mobilization. Therefore, the flow cytometry-based counting of CD34$^+$ cells in the peripheral blood is the most reliable method for initiating apheresis [7–9]. However, because of cost and lack of facilities in most clinical settings, monitoring CD34$^+$ cell number in PB has had little actual effect in avoiding unnecessary aphereses [10,11].

Many transplant centers start apheresis on a fixed day after the initiation of G-CSF administration (e.g., day 4 of G-CSF initiation) because of the low

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probability of failure in stem cell harvest in this setting [12,13]. In other settings, however, the total white blood cell (WBC) count is frequently used as an indicator for the start of apheresis. The best time to begin PBSC collection after the chemotherapy-induced nadir without cytokine support is reported as the day of returning the WBC count to > 1,000/μL [14,15], while the WBC count > 3,000 or > 10,000/μL is recommended when cytokine support is combined [3,16]. The correlation between the WBC count and the number of $CD34^+$ cells in apheresis products, however, is not high, or even non-existent [8,17–20]. Therefore, there exists a need for a good indicator that can be obtained from ordinary clinical data that will reduce the frequencies of failed apheresis. Here, we report a retrospective analysis of the relationship between circulating $CD34^+$ cell number and the level of left shift of granulocytes in the blood.

PATIENTS AND METHODS

Patients

Forty-three consecutive patients with neoplastic diseases who underwent PBSC harvest after chemotherapy followed by G-CSF administration at our institution, between May 1995 and April 2000, were enrolled in the study. Patients with AML were excluded. All patients gave informed consent for the use of a small portion of blood.

Mobilization of PBSC

G-CSF (filgrastim) was given from 2 days after the completion of chemotherapy by single daily subcutaneous administration at 5 μg/kg until a day before the last PBSC apheresis. Apheresis was started using the AS104 continuous-flow blood cell separator (Fresenius AG, St. Wendel, Germany) when the WBC count reached 5,000/μL. At each apheresis, 150–200 mL of blood per kg was subjected to separation. The target cell dose for transplantation was 1×10^6 $CD34^+$ cells/kg actual body weight over 3 consecutive-day aphereses. Patients who failed to achieve the target cell dose underwent remobilization using alternative chemotherapy.

Evaluation for PBSC Graft

The two-color immunofluorescence-staining procedure and flow cytometric analysis have been performed as previously described. The number of $CD34^+$ cells was assessed by the SSC-FL method, i.e., the two-dimensional side scatter-fluorescence representation. The horizontal axis represents fluorescence of $CD34^+$ cells stained with HPCA2-PE antibody (Becton-Dickinson, Mountain View, CA), and the vertical axis represents side scatter. Flow-cytometric analysis was performed on a FACS Caliber (BDIS, San Jose, CA) in fresh samples obtained from each apheresis product. The instrument was operated with CaliBRITE beads (BDIS) and FACSomp software in lyse-wash mode (BDIS). For analysis of hematopoietic progenitor surface antigens in the PBSC harvest, a minimum of 100,000 events for each sample was collected using CellQuest software. In the cases of a large amount of CD34 weak-positive cells included, the cells to be measured were gated in the forward scatter (FCS)-CD45 Didot plot. To accomplish this, larger gates were first set on the CD34 weak-positive region using the CD34-side scatter dot plot. The gates include $CD34^+$ cells, lymphocytes, monocytes, and debris. Next, dual-color analyses of CD34 and CD45 antigens were performed for the gates to distinguish CD45 weak-positive progenitor cells from lymphocytes, monocytes, and debris.

Statistical Analysis

Most statistical analyses were performed with the Stat View statistical package (SAS, Cary, NC). The receiver operating characteristic (ROC) curve [23] was calculated with the SPSS statistical package (SPSS, Chicago, IL).

RESULTS

Patient Characteristics

Characteristics of the 43 patients (29 males, 14 females) included in the study are summarized in Table 1. The median age was 39 years (range, 17–63 years). Twenty-nine patients had hematological malignancies, mostly non-Hodgkin's lymphoma.

PBSC Harvest Data

Thirty-one of 43 patients reached the target $CD34^+$ cell dose of 1×10^6/kg in the first series of mobilizations. In the 12 in whom $CD34^+$ cell dose was $< 1 \times 10^6$/kg, remobilization was performed in five, 4 of whom reached the goal with the second series. The median $CD34^+$ yield was 0.83×10^6/kg (range, $[0.041–22.5] \times 10^6$). The median number of apheresis procedures was 2 (range, 1–4) (Table I).

Predictors of Mobilization and Cell Yield

We next evaluated the relationship between the predictor candidates and $CD34^+$ cell numbers in the respective apheresis products. No correlation was found between $CD34^+$ cell numbers in respective harvests and total WBC count on the day of apheresis ($r = 0.07$, $n = 104$). The percentage of monocytes in WBC was previously reported to have a correlation