was not significantly different (46 vs. 29%; $p = 0.088$). The OS benefit was seen in the patients aged 36–50 years old (49 vs. 24%; $p = 0.031$), suggesting an advantage of allo-HSCT among older patients with leukemia that is more resistant to chemotherapy than that among younger patients.

Keywords AML · Allogeneic hematopoietic stem cell transplantation · Post-remission chemotherapy

1 Introduction

Around 70–80% of newly diagnosed patients with adult acute myeloid leukemia (AML) achieve complete remission (CR) when treated with cytarabine (AraC) and anthracycline, usually daunorubicin (DNR) or idarubicin (IDR). However, only about one-third of these patients remain disease free for more than 5 years [1–5]. Intensified post-remission chemotherapy has improved the survival rates of patients with AML, especially of younger patients [6]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered to be the most intensive post-remission treatment consisting of high-dose chemoradiotherapy and allo-immune mechanisms. However, the powerful anti-leukemic effects of this treatment are counterbalanced by a high incidence of treatment-related mortality (TRM). Thus, allo-HSCT has not always been considered superior to chemotherapy [7, 8]. Intensified chemotherapy with high-dose Ara-C confers promising results on good risk patients [9] for whom allo-HSCT is currently abstained in the first CR (CR1). The Japan Adult Leukemia Study Group (JALSG) AML97 protocol committee circulated a questionnaire among the institutions participating in JALSG regarding their policy about indications for allo-HSCT among AML patients in CR1. The findings revealed that good risk patients in CR1 did not undergo an allo-HSCT at most of these institutions. Cytogenetic profile has been widely used to classify the patients with AML [7–13]; however, cytogenetic studies are not always foolproof. The JALSG established a scoring system that adopted significant factors including cytogenetic results from previous JALSG AML trials [14]. We applied this scoring system to stratify patients and conducted a prospective, multicenter cooperative study (AML97) to compare allo-HSCT with chemotherapy among intermediate and poor risk patients with AML in CR1.

2 Patients and methods

2.1 Patients and study design

The JALSG AML97 study was implemented between December 1997 and July 2001 at 103 institutions where the ethical committees approved the protocol. Adult patients aged from 15 to 64 years newly diagnosed with de novo AML according to the French–American–British (FAB) classification at each institution were eligible, but those with acute promyelocytic leukemia (APL) were excluded. Peripheral blood and bone marrow smears of the registered patients were stained with May-Giemsa, peroxidase, and esterase at Nagasaki University and subsequently reviewed by a central review committee. All patients provided written informed consent to participate before registration in this study.

The chemotherapeutic design of AML97 has been described elsewhere in detail [15]. In short, all the patients were treated with the same induction therapy consisted of AraC (100 mg/m^2, continuous infusion, days 1–7) and IDR (12 mg/m^2 days 1–3). If the patients did not achieve remission after the first induction therapy, then the same therapy was given again. For patients who did not achieve a CR even after second induction therapy, no further treatment was defined in this study. In the comparison between allo-HSCT and chemotherapy as post-remission therapy, these patients were not included in the analysis. All patients who achieved CR were randomized to receive either 4 courses of consolidation therapy without maintenance therapy (group A) or the conventional JALSG post-remission regimen with maintenance therapy (group B) [3]. The results of the two post-remission chemotherapeutic strategies (group A vs. group B) were comparable [15]. The CR patients were classified into good, intermediate or poor risk groups according to the scoring system described below. Intermediate or poor risk patients younger than 50 years old with living siblings were tissue typed. Patients with an HLA-identical sibling were assigned to undergo allo-HSCT soon after three courses of consolidation therapy (donor group), and those without living or HLA-identical siblings were assigned to the no-donor group that continued receiving chemotherapy.

Patients in the donor group with AST or ALT values fourfold higher than the normal range, serum bilirubin and creatinine more than 2 mg/dl, ejection fraction based on an echocardiogram of less than 50% or oxygen saturation according to pulse oximetry of less than 90% were ineligible for allo-HSCT, but were analyzed as a donor group one in an intention-to-treat fashion. Conditioning before transplantation and prophylaxis for graft-versus-host disease was performed according to each institutional standard. Either allogeneic peripheral blood or bone marrow was allowed to be the stem cell source.

2.2 Scoring system

We collected clinical and laboratory data (except for APL) from previous JALSG AML trials (AML87, $n = 234$

Table 1 JALSG scoring system

Scoring system		
System 1		
MPO positive blasts	>50%	+2
Age	≤50 years	+2
WBC	$\leq 2 \times 10^9$/l	+2
FAB subtypes	non-M0, M6, M7	+1
Performance status	0, 1, 2	+1
No. of induction	1	+1
t(8;21) or inv(16)	+	+1
Total score		
Good risk group		8–10
Intermediate risk group		5–7
Poor risk group		0–4
System 2		
MPO positive blasts	>50%	+2
Age	≤50 years	+2
WBC	$\leq 2 \times 10^9$/l	+2
FAB subtypes	non-M0, M6, M7	+1
Performance status	0, 1, 2	+1
Total score		
Good risk group		7–8
Intermediate risk group		4–6
Poor risk group		0–3

MPO myeloperoxidase, *WBC* white blood cell

patients; AML89, $n = 311$; AML92, $n = 986$), and then selected significant factors for achieving CR, disease-free survival (DFS) and overall survival (OS) using multivariate analysis [14]. According to the weight of significance, myeloperoxidase positivity of blasts, patient age, and WBC count at diagnosis were valued at 2 points, and FAB subtypes, performance status, numbers of inductions required to achieve CR, and favorable karyotypes of t(8;21) or inv(16) were valued at 1 point (Table 1, system 1). When we originally planned to use this system, cytogenetic data were not always available at diagnosis. Thus, we designed the system 2 that could be applied even without a cytogenetic data.

2.3 Statistical analysis

The aim of this study was to compare the efficacy of allo-HSCT and chemotherapy as a post-remission treatment, by evaluating DFS and OS rate. Forty-two patients were estimated for an evaluation of the primary endpoint of this study. The JALSG data management committee collected the clinical data from all participating institutions, then fixed them and analyzed the OS of each risk group in July 2004 and the relapse rate (RR), DFS, OS and TRM of the donor and no-donor groups in January 2009. The OS, DFS, RR and TRM were measured from the date of CR. The event for OS was death due to all causes, and patients were censored at the last observation date if alive. The events of DFS were death during CR or relapse. The RR was defined as the cumulative probability of relapse, censoring at death in CR. The events of TRM comprised death before relapse. We estimated OS, DFS, RR and TRM with their respective standard errors using the Kaplan–Meier method [16]. We compared the OS, DFS, RR and TRM between the patients with and without a donor using the log-rank test. Furthermore, the hazard ratio and the 95% confidence interval (CI) of the OS, DFS, RR and TRM were calculated using Cox regression analysis. The Wilcoxon rank-sum test was used for the continuous data, such as age and WBC count, while the Chi-square test was used for the ordinal data, such as the risk group and the frequency of allo-HSCT. All analyses were performed on the intention-to-treat principal with all patients in their allocated arms. Adding to the prospective comparison of the efficacy between allo-HSCT and chemotherapy, we also retrospectively performed subgroup analysis by age. Statistical analyses were conducted using the SAS software package (SAS Institute, Inc, Cary, NC).

3 Results

3.1 Study patients and genetical allocation

Five hundred and three de novo AML patients aged from 15 to 50 years participated in the AML97 comparison of allo-HSCT with chemotherapy as a post-remission therapy. Of 392 patients achieved CR, 62 patients were excluded from the analysis because of insufficient data mainly deficient clinical data at diagnosis which were essential to verify their classification. Three hundred and thirty evaluable patients were classified into the good ($n = 149$), intermediate ($n = 162$) or poor risk ($n = 19$) groups using the scoring system described above (Fig. 1). The 5-year OS

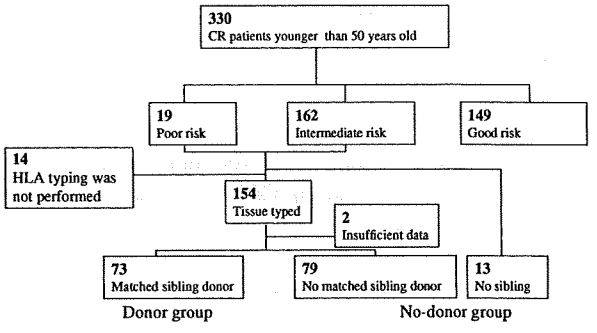

Fig. 1 Overview of patients included in analysis by risk classification, HLA typing, and donor availability

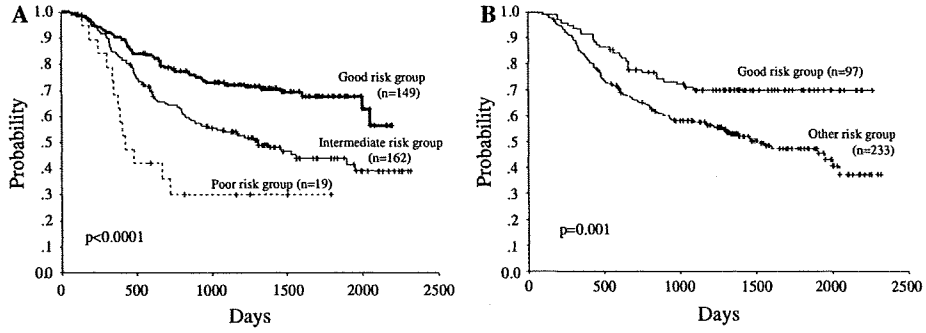

Fig. 2 Overall survival of patients in CR according to JALSG scoring system (**a**) and by cytogenetic studies (**b**)

rates of the CR patients with good, intermediate and poor risk were 68, 44 and 30%, respectively [hazard ratio (HR), 0.51 (good vs. intermediate) and 0.25 (good vs. poor), respectively; 95% confidential interval (CI), 0.35–0.73 (good vs. intermediate) and 0.14–0.48 (good vs. poor); $p < 0.0001$; Fig. 2a]. Among the intermediate and poor risk patients with living siblings, 154 patients and their siblings were examined for their HLA types. Seventy-three of these patients had an HLA-identical sibling and were assigned to the donor group. Thirteen patients with no siblings and 79 patients without an HLA-identical sibling were assigned to the no-donor group (92 patients). Finally, one patient in donor group and one patient in no-donor group were excluded from the analysis because of their insufficient data of survival (Fig. 1). The follow-up durations of the donor and no-donor groups were 1854 days (range 163–3176 days) and 1010 days (range 93–3008 days), respectively.

3.2 Patient characteristics of donor versus no-donor groups

Table 2 shows the characteristics of patients in the donor and no-donor groups. The distributions of these features were comparable in both groups with respect to age, gender, initial WBC count, MPO positivity of blasts, FAB subtype, performance status, prognostic risk according to JALSG score, presence of favorable cytogenetic abnormalities, and the groups of post-remission chemotherapy.

3.3 Donor group

Fifty-six patients (76%) in the donor group actually underwent allo-HSCT (Table 2). Thirty-eight patients (52%) received an allo-HSCT during CR1 at a median of 159 days (range 43–314 days) from CR1. Eighteen patients underwent allo-HSCT after relapse. The median times between CR1 and relapse and between CR1 and a transplantation were 183 days (range 39–757 days) and 248 days (range 157–973 days), respectively. Thirty and 24 patients were transplanted after undergoing a conditioning regimen with or without total body irradiation (TBI), respectively, and conditioning information was not available for 2 patients. The sources of transplanted stem cells were bone marrow cells ($n = 26$), peripheral blood cells ($n = 27$) and bone marrow cells together with peripheral blood cells ($n = 2$). Twenty-nine of the 56 patients in the donor group who underwent allo-HSCT remain alive. Twenty patients died of recurrent leukemia and 7 of transplant-related causes. Seventeen patients allocated to the donor group did not receive a transplantation for the following reasons; patients' refusal ($n = 6$), donors' refusal to donate ($n = 2$), physician's decision ($n = 1$), disease progression before transplantation ($n = 2$), donor health problems ($n = 2$) and unknown reasons ($n = 4$).

3.4 No-donor group

Of the 92 patients in the no-donor group, 42 eventually underwent HSCT (Table 2): autotransplantation ($n = 3$), allo-HSCT from HLA mismatched-related donors ($n = 4$), allo-HSCT from an HLA matched-unrelated donor ($n = 28$), and allo-HSCT from an HLA-mismatched unrelated donor ($n = 7$). Eleven patients underwent a transplantation during CR1 from an unrelated donor or mismatched-related donor at a median of 281 days (range 170–1700 days) from CR1, significantly later than those transplanted during CR1 in the donor group ($p < 0.001$). Thirty-one patients received a transplantation after relapse. The median times between CR1 and relapse and between CR1 and a transplantation were 329 days (range 92–876 days) and 519 days (range 167–1373 days), respectively.

3.5 Comparison of donor versus no-donor groups

The actual risk of relapse at 8 years was significantly lower in the donor group than in the no-donor group (52 vs. 77%, respectively, HR, 0.58; 95% CI, 0.39–0.88; $p = 0.008$; Table 3). The TRM did not significantly differ between the donor and the no-donor groups (16 vs. 17%, respectively, HR, 0.97; 95% CI, 0.34–2.80; $P = 0.959$; Table 3). Seven

Table 2 Patients' characteristics

	Donor	No-donor	*p*
Total number	73	92	
Age			
Median (range)	37 (16–50)	36 (15–50)	0.60[a]
15–35 years	33	46	
36–50 years	40	46	0.54[b]
Sex			
M/F	44/29	45/47	0.15[b]
WBC at diagnosis (10^9/l) (range)	3.8 (0.05–36.8)	5.1 (0.14–45.0)	0.16[a]
MPO positivity of blasts (range)	30 (0–100)	50 (0–100)	0.18[a]
FAB classification			
M0	4	6	
M1	18	25	
M2	22	24	
M4	20	23	
M5	7	14	
M6	1	0	
M7	1	0	0.67[b]
Performance status			
0–1	66	84	
2–3	7	8	0.70[b]
Risk classification by JALSG scoring system			
Intermediate	64	84	
Poor	9	8	0.45[b]
Cytogenetics			
t(8;21) or inv(16)	4	4	0.74[b]
Chemotherapy group			
Group A	38	42	
Group B	30	47	0.28[c]
Not randomized	5	3	
Allogeneic transplant			
During CR1	38	11	
		9 from UD	
		1 from MUD	
		1 from MRD	
After relapse	18	31	
No transplant	17	50	

UD HLA-matched unrelated donor, *MUD* HLA-mismatched unrelated donor, *MRD* HLA-mismatched related donor, *WBC* white blood count, *MPO* myeloperoxidase

[a] Mann–Whitney test

[b] Chi-square test

[c] Chi-square test excluding non-randomized

patients in the donor group and four in the no-donor group died of transplant-related causes during CR1. The lower RR in the donor group resulted in a significantly better DFS compared with the no-donor group (39 vs. 19%, respectively, HR, 0.63; 95% CI, 0.44–0.92; $P = 0.016$; Table 3; Fig. 3). The significant superiority of DFS in the donor group translated into a higher OS rate, but the difference in OS between the two groups did not reach statistical significance (46 vs. 29%, HR, 0.70; 95% CI, 0.47–1.06; $p = 0.088$; Table 3; Fig. 4).

The donor/no-donor analysis was performed on the intention-to-treat principal, which may underestimate the beneficial effect of allo-HSCT probably because of low compliance of transplantation. The 8-year DFS and OS of the recipients actually transplanted during CR1 ($n = 38$) in the donor group were significantly better than those of the patients not transplanted in the no-donor group ($n = 50$); 58 versus 27%, HR, 0.36; 95% CI, 0.20–0.66; $p < 0.001$, and 61 versus 24%, HR, 0.36; 95% CI, 0.19–0.68; $p = 0.001$, respectively.

Table 3 Effects of donor availability on outcome in donor and no-donor groups

Outcome	Donor			No-donor			*p*	HR (95% CI)
	n	No. of events	Probability of outcome at 8 years ±SE (%)	*n*	No. of events	Probability of outcome at 8 years ±SE (%)		
All patients	73			92				
RR		36	52 ± 6		67	77 ± 5	0.008	0.58 (0.39–0.88)
TRM		7	16 ± 6		7	17 ± 7	0.959	0.97 (0.34–2.80)
DFS		44	39 ± 6		74	19 ± 4	0.016	0.63 (0.44–0.92)
OS		37	46 ± 7		61	29 ± 6	0.088	0.70 (0.47–1.06)
Age ≤35	33			46				
RR		17	52 ± 9		31	70 ± 7	0.309	0.74 (0.41–1.33)
TRM		2	12 ± 8		3	15 ± 8	0.785	0.78 (0.13–4.71)
DFS		20	39 ± 9		34	26 ± 7	0.366	0.78 (0.45–1.35)
OS		18	42 ± 10		27	35 ± 9	0.860	0.95 (0.52–1.72)
Age >35	40			46				
RR		19	52 ± 9		36	85 ± 6	0.006	0.46 (0.26–0.81)
TRM		5	19 ± 8		4	19 ± 11	0.962	1.03 (0.27–3.92)
DFS		24	39 ± 8		40	12 ± 5	0.012	0.52 (0.31–0.87)
OS		19	49 ± 9		34	24 ± 7	0.031	0.54 (0.31–0.95)

RR relapse rate, *DFS* disease-free survival, *TRM* treatment-related mortality, *OS* overall survival

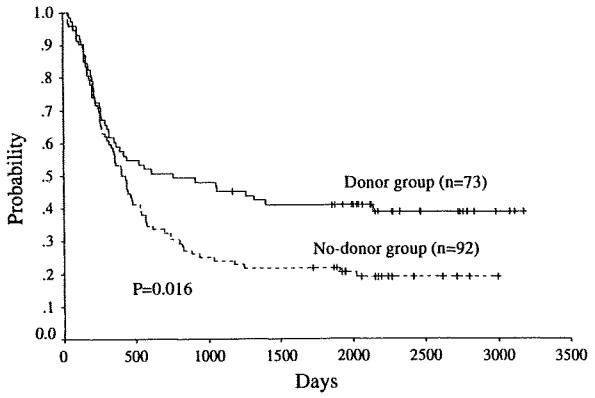

Fig. 3 Disease-free survival in donor and no-donor groups

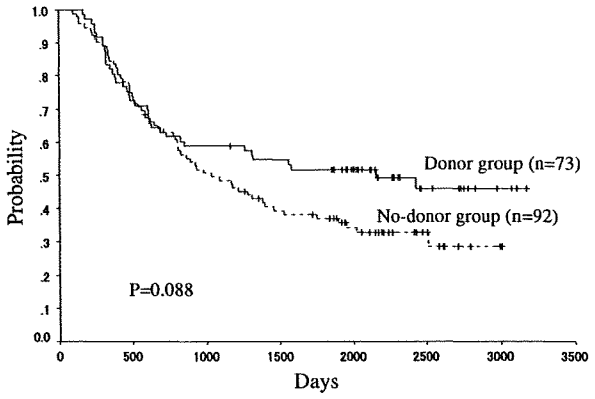

Fig. 4 Overall survival in donor and no-donor groups

3.6 Subset analysis according to patient age

The OS of the patients younger than 35 years of age were comparable between the donor and the no-donor groups (Fig. 5a). However, the OS of the patients aged >35 in the donor group was significantly better compared with the no-donor group (49 vs. 24%, respectively, HR, 0.54; 95% CI, 0.31–0.95; $p = 0.031$; Table 3; Fig. 5b). The RR, TRM, DFS and OS in the donor group were comparable between the two age categories (Table 3; Fig. 5c). In contrast, OS and DFS were marginally worse in the no-donor group of patients aged >35 than ≤35 years (Table 3; Fig. 5d). The distribution of the cytogenetic profile, risk by the JALSG scoring system, myeloperoxidase positivity of blasts, WBC count, FAB classification and performance status at diagnosis did not significantly differ between the two age categories in the no-donor group (data not shown).

4 Discussion

Many clinical trials have compared allo-HSCT with chemotherapy as a post-remission therapy for the patients with AML during CR1. Most of these targeted all patients in CR1 as a single population without prospective stratification by the prognostic factors. Thus, patients were simply assigned into the allo-HSCT or the chemotherapy groups according to donor availability [7, 10, 17, 18]. Here, we

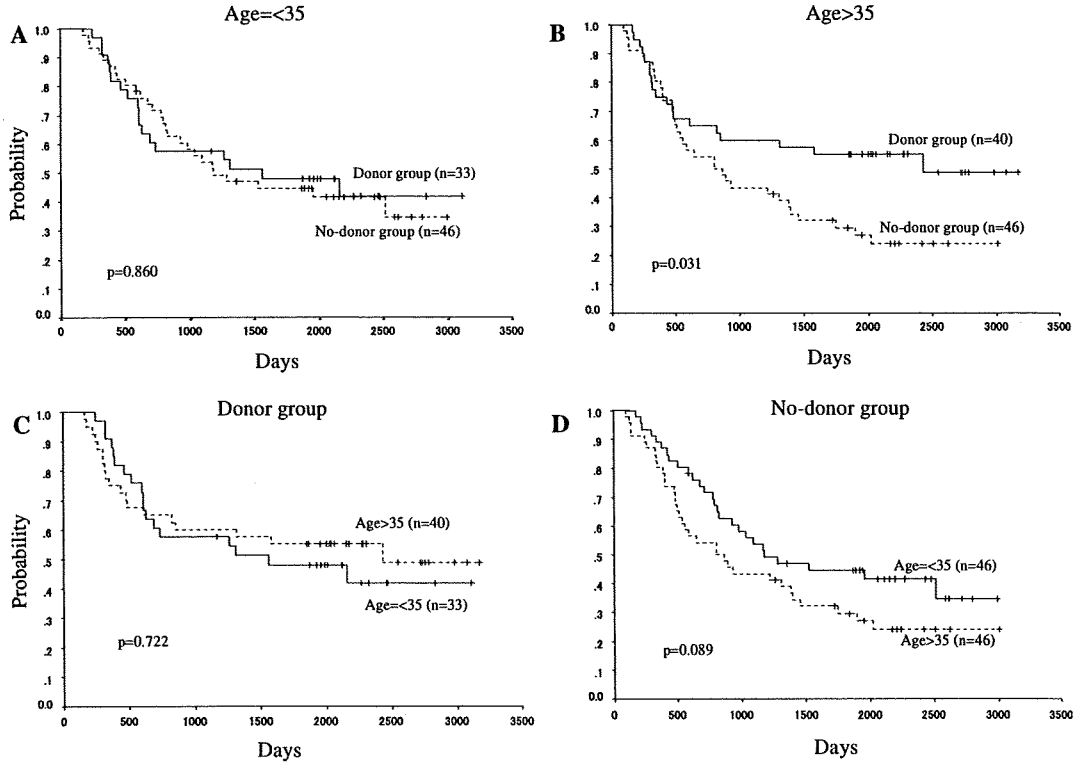

Fig. 5 Overall survival of patients according to age (**a** and **b** ≤35 and >35 years, respectively) and donor availability (**c** and **d**, donor and no-donor groups, respectively)

prospectively compared the effectiveness of allo-HSCT with chemotherapy among patients who were stratified into intermediate or poor risk groups according to JALSG scoring, which constitutes a new means of predicting the prognosis of AML. When this study was planned, as the availability of the cytogenetic study was expected to be variable, and the JALSG scoring system was revealed to be useful to stratify the patients, we adopted a scoring system to select the intermediate and poor risk patients. In contrary to our expectation, cytogenetic studies were performed in 99.2% of the registered patients and the results were available in 97% of the patients. Of 330 CR patients younger than 50 years old, cytogenetic studies disclosed that 97 had good prognostic chromosomal abnormalities, i.e., t(8;21) or inv(16). The OS was significantly better among patients with than without good prognostic cytogenetic profiles (70 vs. 47% at 5 years, with HR, 0.51; 95% CI, 0.34–0.77; $p = 0.001$; Fig. 2b). According to JALSG scoring, 87, 10 and 0 patients with good prognostic cytogenetic abnormalities corresponded to the good, intermediate and poor risk groups, respectively. More good risk patients were selected using this scoring system than by that using karyotype of AML cells alone and about 10% of patients who might be classified into the good risk group by cytogenetic profiles entered the comparison groups by the JALSG scoring system. The JALSG scoring system, which resembles the index used in the Bordeaux Grenoble Marseille Toulouse (BGMT) intergroup study [18], obviously separated patients with a good prognosis who should be excluded from the transplantation trials.

Allo-HSCT prevents AML relapse through intensive cytoreduction using high-dose chemoradiotherapy and graft-versus-leukemia effects. However, previous trials have not always shown advantages of this strategy on the survival of AML patients in CR1. Some studies have not found a benefit of allo-HSCT either on DFS or OS [7, 8], and some showed an advantage only on DFS [10, 17] compared with chemotherapy/auto-transplantation. Retrospective subgroup analysis and meta-analysis have shown a better OS in the donor group [10, 13, 19, 20], demonstrating the importance of limiting the indication of allo-HSCT for only the patients with an intermediate or poor risk.

The following issues should be considered regarding the prospective comparison of allo-HSCT with chemotherapy: assignment of patients according to sibling donor availability [21], low compliance of allo-HSCT for patients in the donor group, and allo-HSCT performed in the no-donor

group from unrelated donors. We could compare the effectiveness of treatment strategies using the intention-to-treat analysis. However, the intrinsic issues of this type of trial and recent advances in alternative stem cell sources will cause difficulties with future prospective comparison of allo-HSCT and chemotherapy using a similar study design.

Although the comparison was performed among patients in the intermediate and poor risk groups, the benefit of allo-HSCT was not significant in OS. Low compliance of allo-HSCT during CR1 in the donor group (52% in the current trial) and allo-HSCT in the no-donor group (total 45%; 11% during CR1) appeared to make the efficacy of allo-HSCT underestimated, especially with regard to OS. However, survival was significantly better among older patients in the donor group (Table 3; Fig. 5b), which seemed to contradict previous findings [19]. Age usually adversely affects allo-HSCT outcome, but it was not associated with the decrease of OS in the donor group in the present study (Table 3; Fig. 5c). Low incidence of TRM probably allowed the powerful anti-leukemic effect of allo-HSCT to function properly, indicating the advantage of allo-HSCT especially among older patients with leukemia that was more resistant to chemotherapy than that among younger patients [1] shown in the no-donor group (Fig. 5d), and caused a contrary result from HOVON/SAKK study. The recent reduction in TRM seemed to contribute much to these results as suggested by others [22, 23]. Different population of the cohorts selected by JALSG scoring and by cytogenetic profiles might also have influenced the present findings.

Molecular markers can be very useful for selecting patients who will most likely benefit from allo-HSCT during CR1 among those with a normal karyotype, which comprises the largest group of patients with AML [24]. The overall safety of allo-HSCT obviously needs improvement, and also patients with chemotherapy-resistant AML who could benefit from allo-HSCT should be identified. Thus, stratification of patients with AML should be improved using a combination of leukemic cell karyotype and, genetic markers and also other clinical findings.

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ORIGINAL ARTICLE

A Phase I/II study of nilotinib in Japanese patients with imatinib-resistant or -intolerant Ph+ CML or relapsed/refractory Ph+ ALL

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Abstract Nilotinib is a second-generation BCR-ABL kinase inhibitor with improved potency and selectivity compared to imatinib. A Phase I/II dose-escalation study was designed to evaluate the efficacy, safety, and pharmacokinetics of nilotinib in Japanese patients with imatinib-resistant or -intolerant Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) or relapsed/refractory Ph+ acute lymphoblastic leukemia (ALL). A total of 34 patients were evaluated in this analysis and had a median duration of drug exposure of 293 (range 13–615) days. All 6 CML-CP patients without complete hematologic response (CHR) at baseline rapidly achieved CHR. A major cytogenetic response was achieved in 94% of patients with CML-CP, including a complete cytogenetic response in 69%. A major molecular response was achieved by 56%. These responses were also observed in patients with CML in advanced stages and Ph+ ALL. Non-hematologic adverse events were mostly mild to moderate. Grade 3 or 4

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neutropenia and thrombocytopenia occurred in 50 and 28% of patients, respectively. Overall, the results of this study suggest that nilotinib induced significant responses in imatinib-resistant or -intolerant patients with CML-CP and CML in advanced stages and Ph+ ALL. The results of this study confirmed the efficacy and safety of nilotinib in Japanese patients.

Keywords Nilotinib · CML · BCR-ABL · Imatinib resistant · Ph+ ALL

1 Introduction

The Philadelphia chromosome (Ph), which results from a reciprocal translocation between the long arms of chromosomes 9 and 22, is detected in more than 90% of chronic myeloid leukemia (CML) and 20–30% of adult acute lymphoblastic leukemia (ALL). The Philadelphia chromosome carries the *BCR-ABL* fusion gene, which encodes a constitutively active protein tyrosine kinase [1, 2]. Without BCR-ABL-targeted therapy, CML generally progresses within several years from a stable chronic phase (CP) to an accelerated phase (AP), and terminates in blast crisis (BC) [3]. Ph+ ALL is the most aggressive form of ALL and carries a poor prognosis comparable to CML-BC [4].

Imatinib (Gleevec®, Glivec®; Novartis Pharmaceuticals, Florham Park, NJ), a BCR-ABL tyrosine kinase inhibitor (TKI), has greatly improved the outcome in CML. In newly diagnosed CML patients, treatment with imatinib has shown a complete cytogenetic response (CCyR) rate of 87%, a progression rate to AP or BC of 7%, and an estimated 5-year survival rate of 89% [5, 6]. At the 6-year follow-up, the CCyR rate was 82% with 0% transformation to AP or BC between years 5 and 6, and an estimated 6-year overall survival of 88% [7]. However, resistance and intolerance to imatinib does occur in some patients and, therefore, additional treatment options are necessary to address these unmet medical needs.

Nilotinib (Tasigna®; Novartis Pharmaceuticals) is a second-generation TKI with improved potency and target specificity [8]. Like imatinib, nilotinib binds to and stabilizes an inactive conformation of the kinase domain of the ABL protein, thus preventing the enzyme from adopting the catalytically active conformation and blocking the tyrosine phosphorylation of proteins involved in BCR-ABL signal transduction [8, 9]. Nilotinib has been approved for the treatment of patients with CML-CP and -AP resistant to or intolerant of prior therapy, including imatinib, in 50 countries, including the United States and Europe based on the pivotal Phase II registration study. Responses to nilotinib were rapid and durable, with the vast majority of patients with CML-CP or -AP remaining alive at 12 months. Nilotinib is generally well tolerated, with a minimal occurrence of grade 3/4 drug-related adverse events and a favorable hematologic adverse event profile compared to other second-generation TKIs. Nilotinib also displayed significant activity in imatinib-resistant or -intolerant CML-BC, and relapsed/refractory Ph+ ALL, with significant rates of complete hematologic response (CHR), major cytogenetic response (MCyR), and CCyR [10–14].

This Phase I/II dose-escalation study, including an extension portion of the study, was conducted to confirm the efficacy, safety, and pharmacokinetic profiles of nilotinib in Japanese patients with imatinib-resistant or -intolerant CML or relapsed/refractory Ph+ ALL.

2 Methods

2.1 Study design and patient population

A Phase I/II dose-escalation study with an extension portion of the study was designed to evaluate the efficacy, safety, and pharmacokinetics of nilotinib. Patients who completed at least three 28-day cycles of treatment in the Phase I/II study without discontinuation were enrolled into the extension study. Tolerability up to the dose levels 400 mg BID, clinical dose approved in the US and Europe was confirmed in Japanese patients in a Phase I component [15].

Japanese patients were eligible for this multi-center, open label study when having imatinib-resistant/intolerant Ph+ CML or relapsed/refractory Ph+ ALL who were at least 20 years of age. Patients also needed to have adequate performance status (World Health Organization [WHO] Performance Score [PS] $\leq$ 2) and normal hepatic, renal, and cardiac functions.

CML-CP, -AP, -BC, Ph+ ALL and Ph+ ALL with minimal residual disease (MRD) were defined as previously described [10–14]. Imatinib resistance in patients with CML-CP was defined as failure to achieve CHR after 3 months, cytogenetic response (CyR) after 6 months, MCyR after 12 months, or loss of a hematologic or cytogenetic response at any time during treatment with imatinib following a minimum of 3 months of imatinib therapy with at least 600 mg/day. Imatinib resistance for CML-AP and -BC was defined by one of the following criteria during treatment with at least 600 mg/day of imatinib: (1) disease progression from chronic phase to accelerated or blast phase occurring during imatinib therapy; (2) disease progression defined as at least a 50% increase in peripheral white blood cell count, blast count, basophils, or platelets during imatinib therapy for accelerated or blast phase; or (3) lack of hematologic response (HR) in the bone marrow following a minimum of 4 weeks of imatinib therapy for

accelerated or blast phase. In addition, patients receiving less than 600 mg/day of imatinib were eligible for participation if *BCR-ABL* mutations were found present by sequencing any one of the following amino acids: L248, G250, Q252, Y253, E255, T315, F317, H396, M237, M244, D325, S348, M351, E355, A380, L387, M388, F486, and F359.

Imatinib intolerance for CML patients was defined as the discontinuation of imatinib therapy due to any of the following: grade 3 or 4 adverse events that persisted in spite of optimal supportive care measures, or grade 2 adverse events related to imatinib therapy in spite of optimal supportive care measures that persisted for at least 1 month or that recurred more than 3 times whether the dose was reduced or discontinued. In addition, the protocol definition of imatinib intolerance included the lack of a MCyR with imatinib.

Nilotinib (400 mg) was administered twice daily (BID; every 12 h) with water, while fasting 2 h before and 2 h after dosing. Dose reductions to 400 mg daily and subsequently 200 mg daily were permitted for the management of toxicity. If administration of a dose was delayed for more than 21 days for the management of toxicity (or more than 42 days for grade 3 or 4 hematologic toxicity), the patient was discontinued from the study. Treatment with nilotinib was continued until the patient experienced disease progression, developed unacceptable toxicity that precluded any further treatment, withdrew consent, and/or if the patient no longer benefited from the treatment (at the investigator's discretion).

2.2 Statistical analysis

The intent-to-treat (ITT) population and the safety population were included in the efficacy (including analyses for biomarker) and the safety analyses, respectively. The ITT population included all patients who received at lease one dose of nilotinib 400 mg BID. The safety population included all the patients in the ITT population who had at least one safety assessment. Pharmacokinetic analyses were performed for the pharmacokinetic population that included all patients who had available pharmacokinetic sample data. All analyses presented in this paper are based on the data obtained with the cut-off date of 3 October 2007 in all patients who received nilotinib 400 mg BID in any component of the study, including the Phase I, II, and its extension portions.

The rates on overall best hematologic response were summarized by disease phases and type (CML-CP, -AP, -BC, relapsed/refractory Ph+ ALL, and Ph+ ALL with MRD). The rates on overall best cytogenetic response were summarized for all CML patients. For CML-CP group only, 95% confidence intervals (95% CIs) using Clopper–Pearson limits were determined. Other efficacy analyses included the time-to-first response and duration of response using either the descriptive statistics or the Kaplan–Meier method.

2.3 Efficacy parameters

Hematologic and cytogenetic response criteria have been described in detail previously [10–14]. Criteria for cytogenetic responses are as follows: complete (0% Ph+ cells), partial (1–35% Ph+ cells), minor (36–65% Ph+ cells), and minimal (66–95% Ph+ cells). A MCyR includes both complete and partial cytogenetic responses. Cytogenetic responses were based on the percentage of Ph+ cells among 20 or more cells in metaphase in each bone marrow sample. Results obtained from fluorescent in situ hybridization (FISH) were also used to determine cytogenetic response only if fewer than 20 cells in metaphase were examined or bone marrow sample was not adequate on a particular assessment date due to other reasons. Only evaluable patients in the ITT population were included in the analysis for overall best hematologic and cytogenetic response rates. Patients with Ph+ CML who had a CHR at baseline were not included in the efficacy analysis for best hematologic response rates. Similarly, Ph+ CML patients who had CCyR at baseline were excluded from the analysis for best cytogenetic response. Evaluable patients who discontinued the study with no valid efficacy assessment were not included in the analysis for best responses.

2.4 Biomarkers

Peripheral blood samples were obtained prior to the first dose of nilotinib and every 3 months during nilotinib therapy. The BCR-ABL kinase domain (amino acid 230–490) was amplified from total blood RNA and mutations identified by direct sequencing that allowed for detection of more than 20% minor alleles. BCR-ABL transcript levels in blood were also monitored by a real-time quantitative RT-PCR (qRT-PCR) assay. The BCR-ABL mutational and qRT-PCR analyses were performed by Institute of Medical and Veterinary Science, Adelaide, Australia.

Patients were grouped based on their baseline mutational status: no mutation, any mutation, or multiple mutations. The number and percentage of patients who achieved a HR and CyR and major molecular response (MMR) were calculated for each mutation category in order to investigate the correlation between clinical responses and baseline BCR-ABL mutation status. A MMR was defined as BCR-ABL/control gene ratio of $\leq 0.1\%$ based on international scale, equivalent to ≥ 3 log reduction in BCR-ABL transcripts from the standardized baseline as determined in the international randomized study of

interferon and STI571 (IRIS) study. The number and percentage of patients who had at least one MMR post-baseline were calculated by disease phase. Patients who had a MMR at baseline were excluded from the analysis.

2.5 Safety parameters

Safety assessments included evaluation of adverse events, hematologic and biochemical testing, urinalysis, cardiac enzyme assessment, blood coagulation test, WHO PS scores, vital signs, physical examinations, 12-lead ECG, echocardiography, and chest X-rays. All adverse events were recorded with grades based on the Common Terminology Criteria for Adverse Events (CTCAE, version 3.0) of the National Cancer Institute, and monitored for at least 28 days after the last dose of nilotinib in patients who discontinued the study. Laboratory measurements were evaluated based mainly on the calculated CTC grades at baseline and post-baseline.

2.6 Pharmacokinetic parameters

The pharmacokinetic parameters were calculated by the standard non-compartmental method using WinNonlin Professional Edition 5.0 (Pharsight Corporation). Serum concentrations of nilotinib below the limit of quantitation (2.5 ng/mL) were treated as zero for the calculation of pharmacokinetic parameters. The following parameters were obtained: maximum serum concentration of nilotinib (C_{max}), time to reach C_{max} (T_{max}), area under the serum nilotinib concentration time curve from time 0 to 12 h post-dosing (AUC_{0-12}), and minimum serum concentration of nilotinib (C_{min}), defined as the concentration immediately before nilotinib administration.

2.7 Study conduct

The study was conducted in accordance with the Declaration of Helsinki. Patients gave written informed consent, according to institutional guidelines. The study was approved by the institutional review board at each study center.

3 Results

3.1 Patient demographics

Results are presented for 34 patients with at least 12 months of follow-up or those who prematurely discontinued study treatment. These include 31 patients enrolled in the Phase II component of the study (14 CML-CP, 7 CML-AP, 3 CML-BC, 7 Ph+ ALL) and 3 patients enrolled in the Phase I component of the study who received nilotinib 400 mg BID (2 CML-CP, 1 CML-BC). Disposition of patients is shown in Table 1. Of the 34 patients, 25 were enrolled in the extension study (16 CML-CP, 3 CML-AP, 3 CML-BC, 3 Ph+ ALL). At the time of data cut-off, 17 (50%) patients remained in the study. The most frequent reason for treatment discontinuation was disease progression.

The median duration of exposure (293 days; range 13–615) closely approximates the median duration of treatment (291 days; range 13–615) indicating minimal duration of treatment interruption. Nilotinib was well tolerated as indicated by the administration of median dose intensity (756 mg/day; range 285–799) which was close to the planned dose (400 mg BID = 800 mg/day) for the study.

Demographic and other baseline characteristics of patients are shown in Table 2. The median age of all

Table 1 Disposition of patients (ITT population)

	n (%)				
	CML-CP $N = 16$	CML-AP $N = 7$	CML-BC $N = 4$	Ph+ ALL $N = 7$	Total $N = 34$
Patients who enrolled in the extension study	16 (100)	3 (43)	3 (75)	3 (43)	25 (74)
Patients with treatment ongoing at cut-off date	15 (94)	1 (14)	0 (0)	1 (14)	17 (50)
Discontinued treatment at cut-off date	1 (6)	5 (71)[a]	4 (100)	6 (86)	16 (47)[a]
Reason for discontinuation					
Adverse event(s)	0 (0)	1 (14)	1 (25)	1 (14)	3 (9)
alloHSCT performed	1 (6)	2 (29)	1 (25)	0 (0)	4 (12)
Disease progression	0 (0)	2 (29)	2 (50)	5 (71)	9 (27)

The ITT population included all patients in the Phase I and Phase II studies who were administered a 400 mg BID dose at least once

alloHSCT allogeneic hematopoietic stem cell transplantation

[a] Does not include one patient who completed three cycles of the initial therapy but did not move to the extension study

Table 2 Demographic and other baseline characteristics (ITT population)

	CML-CP $N = 16$	CML-AP $N = 7$	CML-BC $N = 4$	Ph+ ALL $N = 7$	Total $N = 34$
Age (years)					
Median	57	61	53	62	62
Range (min–max)	30–83	30–74	29–70	23–80	23–83
Sex, *n* (%)					
Male	9 (56)	5 (71)	2 (50)	6 (86)	22 (65)
Female	7 (44)	2 (29)	2 (50)	1 (14)	12 (35)
Weight (kg)					
Median	61	65	63	56	61
Range (min–max)	45–89	49–83	36–69	46–60	36–89
WHO performance status, *n* (%)					
Grade 0	16 (100)	4 (57)	2 (50)	4 (57)	26 (76)
Grade 1	0 (0)	2 (29)	2 (50)	3 (43)	7 (21)
Grade 2	0 (0)	1 (14)	0 (0)	0 (0)	1 (3)
Grade >2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Time since first diagnosis (months)					
<6 months	2 (13)	0 (0)	0 (0)	1 (14)	3 (9)
≥6 months to <1 year	2 (13)	0 (0)	0 (0)	1 (14)	3 (9)
≥1 year to <2 years	3 (19)	2 (29)	1 (25)	4 (57)	10 (29)
≥2 years to <5 years	3 (19)	0 (0)	1 (25)	0 (0)	4 (12)
≥5 years	6 (38)	5 (71)	2 (50.0)	1 (14)	14 (41)
Number of patients, *n* (%)					
Imatinib resistant	4 (25)	4 (57)	4 (100)	7 (100)	19 (56)
Imatinib intolerant	12 (75)	3 (43)	0 (0)	0 (0)	15 (44)
Highest imatinib dose					
Mean ± SD	519 ± 210	686 ± 157	700 ± 115	600 ± 0	591 ± 178
Median	500	800	700	600	600
Range (min–max)	200–800	400–800	600–800	600–600	200–800

patients was 62 years (range 23–83 years), and approximately 65% of all patients were male. The median weight of all patients was 61 kg (range 36–89 kg). Imatinib-intolerant patients constituted 75% (12/16) of CML-CP, though all CML-BC and Ph+ ALL patients were imatinib resistant. One of 4 imatinib-resistant CML-CP patients was primary resistant.

3.2 Pharmacokinetic analysis

Table 3 shows pharmacokinetic parameters determined following the administration of nilotinib 400 mg BID. Absorption of nilotinib was relatively rapid with median T_{max} of 3 h with large inter-individual variability. Steady state was achieved by day 6 after repeated dosing since C_{min} had been almost constant after day 6. The accumulation ratio calculated by ratio of AUC_{0-12} on days 15 to 1 was 2.64 ± 1.07 (mean ± SD). The serum concentrations of nilotinib did not differ among the phases of CML and Ph+ ALL group on the first day of treatment. On day 15, nilotinib exposure in the CML-BC group and Ph+ ALL group appeared to be slightly higher than in other groups, but this difference is most likely due to the small patient number and relatively large inter-individual variability. Steady-state nilotinib concentrations observed on day 15 in this study were similar to those observed previously in non-Japanese patients [16].

3.3 Efficacy

3.3.1 Hematologic response

Table 4 details the HR rates. Six of the 16 patients with CML-CP patients without a CHR at baseline were included in the efficacy analysis for HR. All 6 patients achieved a CHR (100%; 95% CI: 54.1–100.0%). In the CML-AP patients, a HR was achieved in 5/7 (71%) patients, including 1 CHR, 3 marrow responses with no evidence of leukemia (NEL), and 1 return to CP. In the CML-BC patients, a HR was achieved in 2/4 (50%) patients,

Table 3 Pharmacokinetic parameters following administration of 400 mg BID of nilotinib (ITT population)

	N	T_{max} (h) [median (range)]	Mean ± SD		
			C_{max} (ng/mL)	AUC_{0-12} (ng h/mL)	C_{min} (ng/mL)
Day 1					
Total	33	3.0 (2.0–23.0)	1070 ± 458	7850 ± 2790	NA
CML-CP	15	3.0 (2.0–7.0)	942 ± 276	7110 ± 1800	NA
CML-AP	7	3.0 (2.9–23.0)	1120 ± 614	7550 ± 3150	NA
CML-BC	4	5.5 (3.0–7.0)	1150 ± 458	8880 ± 2700	NA
Ph+ ALL	7	3.0 (2.2–7.0)	1220 ± 618	9150 ± 4010	NA
Day 15					
Total	28	3.0 (1.8–8.0)	2320 ± 1070	19000 ± 9090[a]	1170 ± 588
CML-CP	13	3.0 (1.9–8.0)	2010 ± 652	17200 ± 6030[b]	1051 ± 410
CML-AP	6	3.0 (1.8–3.0)	1760 ± 884	15000 ± 6770	885 ± 349
CML-BC	3	2.1 (1.9–5.0)	3210 ± 1340	30300 ± 15200	1890 ± 893
Ph+ ALL	6	3.0 (1.9–8.0)	3140 ± 1310	21200 ± 10400[c]	1350 ± 732

NA not applicable

[a] $N = 26$

[b] $N = 12$

[c] $N = 5$

including 1 CHR and 1 return to CP. The rate of HR confirmed at 2 consecutive visits at least 4 weeks apart was achieved in 2/7 (29%) CML-AP patients, 2/4 (50%) CML-BC patients. A CR was achieved in 1/5 (20%) relapsed/refractory Ph+ ALL and both 2 Ph+ ALL patients with MRD.

The median time to CHR was 1 month (range 1–2 months) for CML-CP patients. Time to HR was 1 month for all of the CML-AP, BC and Ph+ ALL patients but 2 months in one CML-BC patient. The duration of CHR was not determined because all CML-CP patients were still responding to treatment at the data cut-off date. The range of duration of CHR in 6 CML-CP patients who achieved CHR up to the data cut-off date was 11.6–13.6 months. All ten patients with CHR at baseline were maintaining response at the time of data cut-off. In 3 of the 5 patients with CML-AP who achieved a HR, the response continued until either the time they discontinued study treatment or the data cut-off date. The duration of the other 2 CML-AP patients was 1 and 2 months each. Of the 2 patients with CML-BC who achieved a HR, one was still in HR at data cut-off and one had a duration of HR of 2 months. For patients with relapsed/refractory Ph+ ALL, 1 who achieved a HR continued to show a response for 3.9 months. One of the two Ph+ ALL patients with MRD was still in HR at data cut-off and the other lasted for 2 months.

3.3.2 Cytogenetic response

A MCyR was achieved in 15 of the 16 CML-CP patients (94%; 95% CI: 70–100.0%), and a CCyR in 11 (69%) of these patients (Table 5). A CCyR was achieved in 1 (14%) of the 7 CML-AP patients. In the other 6 patients, 3 achieved a minimal CyR, 1 no CyR, and 2 patients were considered not assessable for response due to dry-tap bone marrow. As for the patients with CML-BC, 2 of the 4 patients (50%) achieved a CCyR.

Time to MCyR or CCyR was evaluated in CML-CP patients. The median time to MCyR was 3 months (range 1–6.6 months) and the time to CCyR was also 3.2 months (range 2–11.9 months). The MCyR continued, in all patients achieving MCyR, until the data cut-off date or discontinuation from study treatment, so the median duration of MCyR has not been reached at the time of data cut-off.

3.3.3 Molecular response

A MMR was achieved in 9 (56%) of 16 CML-CP patients. The median time to MMR was 6.3 months (range 3–18.3 months). These 9 patients are still in MMR at data cut-off. A MMR was achieved in 1 (14%) of the 7 CML-AP patients, 2 (50%) of the 4 CML-BC patients. None achieved MMR in relapsed/refractory Ph+ ALL. One of 2 Ph+ ALL with MRD achieved MMR and the other one was considered as not evaluable due to MMR at baseline.

3.3.4 Response by BCR-ABL mutation status

BCR-ABL mutations at baseline were detected in 4 (25%) of 16 CML-CP patients, in 6 (86%) of 7 CML-AP patients, in 2 (50%) of 4 CML-BC patients, and in 4 (57%) of 7 Ph+ ALL patients. A total of 14 different BCR-ABL mutations

Table 4 Best hematologic response (ITT population)

Disease	Evaluation criteria	*n* (%)
CML-CP ($N = 16$)	Not evaluable	10 (63)
	Evaluable	6 (38)
	Complete hematologic response	6 (100)
	Stable disease	0
	Progression of disease	0
	Not assessable	0
CML-AP ($N = 7$)	Hematologic response	5 (71)
	Complete hematologic response	1 (14)
	Marrow response with no evidence of leukemia	3 (43)
	Return to chronic phase	1 (14)
	Stable disease	1 (14)
	Progression of disease	0
	Not assessable	1 (14)
CML-BC ($N = 4$)	Hematologic response	2 (50)
	Complete hematologic response	1 (25)
	Marrow response with no evidence of leukemia	0
	Return to chronic phase	1 (25)
	Stable disease	2 (50)
	Progression of disease	0
	Not assessable	0
Relapsed/refractory Ph+ ALL ($N = 5$)	Hematologic response	1 (20)
	Complete response	1 (20)
	Partial response	0
	Hematologic improvement	0
	Stable disease	1 (20)
	Progression of disease	3 (60)
	Not assessable	0
Ph+ ALL with minimal residual disease ($N = 2$)	Complete response	2 (100)
	Stable disease	0
	Progression of disease	0
	Not assessable	0

involving 12 amino acids were detected. There were no patients with a T315I mutation, which is known to cause imatinib and nilotinib resistance. HR and CyR could be observed in patients with any of the disease stages who were administered nilotinib, regardless of BCR-ABL mutation status and regardless of their specific mutation (Table 6).

3.3.5 Safety

Non-hematologic adverse events that were suspected to be related to nilotinib are summarized in Table 7. These were

Table 5 Cytogenetic response (Ph+ CML, ITT population)

	n (%)		
	CML-CP $N = 16$	CML-AP $N = 7$	CML-BC $N = 4$
Evaluable	16 (100)	7 (100)	4 (100)
Major CyR	15 (94)	1 (14)	2 (50)
Complete	11 (69)	1 (14)	2 (50)
Partial	4 (25)	0	0
Minor CyR	0	0	1 (25)
Minimal CyR	1 (6)	3 (43)	0
None	0	1 (14)	0
Not assessable	0	2 (29)	1 (25)

mostly mild to moderate in severity. The most commonly reported events were rash (50%), headache (32%), nausea (32%), vomiting (29%) and pyrexia (24%); however, grade 3 or higher events were uncommon.

The numbers of patients with newly occurring or worsening grade 3 or 4 laboratory abnormalities are summarized in Table 8. Grade 3 or 4 abnormalities in neutropenia and thrombocytopenia occurred in 50 and 28% of patients, respectively. These hematologic abnormalities were generally manageable with dose interruptions and reductions, and support with hematopoietic growth factors or transfusions occasionally. Only one patient discontinued from study treatment due to thrombocytopenia.

The majority of biochemistry abnormalities were mild to moderate in severity, resolved spontaneously with continued dosing of nilotinib. Grade 3 or 4 elevations in AST and ALT occurred in 6 and 12% of patients, respectively. Grade 3 or 4 total bilirubin occurred in 3%. Grade 3 or 4 elevations of lipase occurred in 15% of patients. Pancreatitis was reported in 1 patient; however, it was transient and resolved with dose interruption and reduction. No patients discontinued therapy due to serum biochemistry abnormalities. Gastrointestinal and central nervous system hemorrhage of grade 3 or 4 was not reported.

One death was occurred in the study, or within 28 days of discontinuing study. The patient discontinued study treatment because of back pain on study day 14 and died as a result of cardiac failure due to cardiac tamponade and pericardial effusion on day 16. Grade 3 or 4 peripheral edema, pericardial effusion, or pleural effusion was not reported in other patients. Because of a preclinical signal indicating that nilotinib could potentially prolong the QT interval, frequent ECG was performed during the study. One patient experienced a prolongation in the QTcF interval exceeding 500 ms. This event resolved spontaneously with dose reduction of nilotinib. No episodes of torsades de pointes were observed. No tendency was observed for the incidence of adverse events to increase or

Table 6 Hematologic response and cytogenetic response by BCR-ABL mutation at baseline (ITT population)

Disease type	Mutation	Hematologic response		Major cytogenetic response		Major molecular response	
		N^a	*n* (%)	N^b	*n* (%)	N^c	*n* (%)
CML-CP ($N = 16$)	No mutation	4	4 (100)	12	12 (100)	12	6 (50)
	Any mutation	2	2 (100)	4	3 (75)	4	3 (75)
	D276G	1	1 (100)	1	1 (100)	1	1 (100)
	M244V	1	1 (100)	1	0 (0)	1	0 (0)
	F359I	–	–	1	1 (100)	1	1 (100)
	F311I	–	–	1	1 (100)	1	1 (100)
CML-AP ($N = 7$)	No mutation	1	1 (100)	1	0 (0)	1	0 (0)
	Any mutation	6	4 (67)	6	1 (17)	6	1 (17)
	M351T	1	1 (100)	1	0 (0)	1	0 (0)
	Y253H	2	1 (50)	2	0 (0)	2	0 (0)
	F359I/L387 M	1	1 (100)	1	1 (100)	1	1 (100)
	F311I/M244V	1	1 (100)	1	0 (0)	1	0 (0)
	E279K	–	–	–	–	1	0 (0)
CML-BC ($N = 4$)	No mutation	2	1 (50)	2	1 (50)	2	1 (50)
	Any mutation	2	1 (50)	2	1 (50)	2	1 (50)
	F317L	1	1 (100)	1	1 (100)	1	1 (100)
	F359V	1	0 (0)	1	0 (0)	1	0 (0)
Ph+ ALL ($N = 7$)	No mutation	3	2 (67)	–	–	3	1 (33)
	Any mutation	4	1 (25)	–	–	3	0 (0)
	E255K, V/G250E	1	0 (0)	–	–	1	0 (0)
	E459K	1	0 (0)	–	–	1	0 (0)
	E255V	1	0 (0)	–	–	1	0 (0)
	F359V	1	1 (100)	–	–	–	–

[a] Number of patients deemed to be evaluable for hematologic response when an analysis of BCR-ABL mutations was performed post-baseline

[b] Number of patients deemed to be evaluable for cytogenetic response when an analysis of BCR-ABL mutations was performed post-baseline

[c] Number of evaluable patients to be included in the mutation category, i.e., patients who had mutation data and who did not have MMR at baseline

for their onset to be delayed as treatment with the study drug continued.

4 Discussion

Imatinib, the first BCR-ABL TKI approved for the treatment of Ph+ CML and Ph+ ALL, has demonstrated clinical efficacy. However, resistance develops in some patients and treatment options for patients who are resistant to, or intolerant of, imatinib have been very limited. Nilotinib is a more potent and more selective inhibitor of the BCR-ABL protein tyrosine kinase.

The results of this study show the high level of clinical activity of nilotinib in Japanese patients with CML and Ph+ ALL as the overseas Phase II registration study. The rates of CHR and MCyR were relatively higher than that of overseas data. However, due to the limited data in Japanese patients, it is difficult to draw a meaningful conclusion. Overall, imatinib resistance or intolerance, and baseline BCR-ABL mutation status, did not appear to have an impact on response to nilotinib.

The most frequent drug-related adverse events were rash, headache, nausea, vomiting, and pyrexia; however, grade 3 or 4 events were uncommon. Grade 3 or 4 peripheral edema or pleural effusion was not reported. Though neutropenia and thrombocytopenia occurred in 50 and 28% of patients, respectively, these were generally manageable with dose interruptions and reductions, and support with hematopoietic growth factors or transfusions occasionally. Hemorrhage of grade 3 or 4 was not reported. The majority of serum biochemistry abnormalities were infrequent, and mild to moderate in severity, resolved spontaneously with continued dosing of nilotinib.

Although imatinib intolerance constituted 75% (12/16) in CML-CP patients, no patient experienced same serious side effects or side effects lead to discontinuation of administration of nilotinib. Nilotinib and imatinib have some structural features in common, but the minimal occurrence of cross-intolerance between the 2 agents may represent significant therapeutic advantage. It is important to note that no imatinib-intolerant patient on this study had achieved a prior CyR on imatinib at any time.

Table 7 Non-hematologic adverse events suspected to be related to nilotinib (10% or more, SAF population)

	Total ($N = 34$)			
	All grades		Grade 3/4	
	n	%	*n*	%
Rash	17	50	1	3
Headache	11	32	2	6
Nausea	11	32	1	3
Vomiting	10	29	0	0
Pyrexia	8	24	0	0
Malaise	5	15	0	0
Hepatic function abnormal	5	15	0	0
Anorexia	5	15	0	0
Eczema	5	15	0	0
Constipation	4	12	0	0
Stomach discomfort	4	12	0	0
Chest pain	4	12	0	0
Back pain	4	12	1	3
Muscle spasms	4	12	0	0
Erythema	4	12	0	0
Pruritus	4	12	0	0

Consistent with previous findings, the pharmacokinetic profile of nilotinib showed moderate inter-individual variability in this study with Japanese CML patients. The observed variation in nilotinib pharmacokinetics may be partly attributed to the inter-individual variability in CYP3A4, since CYP3A4 activity has been shown to vary among different individuals and nilotinib is mainly metabolized by CYP3A4. Pharmacokinetic parameters obtained in this study were similar with those in non-Japanese patients, indicating that there would be no ethnic difference in pharmacokinetic profile of nilotinib [16]. Thus, the mean steady-state plasma trough level of 1170 mg/mL (2.2 μM) represents a concentration sufficient to inhibit the in vitro proliferation of most cell lines expressing imatinib-resistant mutant forms of BCR-ABL (with the exception of the T315I mutation) [9].

In summary, nilotinib is highly active and safe, and provides an effective treatment for Japanese patients with Ph+ CML whose disease becomes resistant or intolerant to imatinib. Promising activity is also observed in relapsed/refractory Ph+ ALL patients.

Table 8 Newly occurring or worsening grade 3/4 laboratory abnormalities (SAF population)

	n/N (%)				
	CML-CP $N = 16$	CML-AP $N = 7$	CML-BC $N = 4$	Ph+ ALL $N = 7$	Total $N = 34$
Hematology					
WBC	5/16 (31)	3/7 (43)	3/4 (75)	3/6 (50)	14/33 (42)
Neutrophils	6/16 38)	5/7 (71)	2/3 (67)	3/6 (50)	16/32 (50)
Lymphocyte	6/16 (38)	1/7 (14)	2/4 (50)	2/6 (33)	11/33 (33)
PLT	3/16 (19)	2/6 (33)	2/4 (50)	2/6 (33)	9/32 (28)
Hemoglobin	3/16 (19)	4/7 (57)	4/4 (100)	4/7 (57)	15/34 (44)
Biochemistry					
ALP	0/16 (0)	0/7 (0)	0/4 (0)	0/7 (0)	0/34 (0)
AST (GOT)	0/16 (0)	0/7 (0)	1/4 (25)	1/7 (14)	2/34 (6)
ALT (GPT)	2/16 (13)	0/7 (0)	1/4 (25)	1/7 (14)	4/34 (12)
Bilirubin (total)	1/16 (6)	0/7 (0)	0/4 (0)	0/7 (0)	1/34 (3)
Amylase	1/16 (6)	0/7 (0)	0/4 (0)	0/7 (0)	1/34 (3)
Lipase	4/16 (25)	1/7 (14)	0/4 (0)	0/7 (0)	5/34 (15)
Phosphate (hypo)	2/16 (13)	1/7 (14)	1/4 (25)	1/6 (17)	5/33 (15)
Glucose (hyper)	2/16 (13)	1/7 (14)	0/4 (0)	0/7 (0)	3/34 (9)
Glucose (hypo)	0/16 (0)	0/7 (0)	0/4 (0)	0/7 (0)	0/34 (0)

Patients are counted only for the worst grade observed post-baseline

n number of patients who had less than grade *X* at baseline, and worsened to grade *X* post-baseline; *N* total number of patients evaluable post-baseline who had less than grade *X* at baseline

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Differences in the distribution of subtypes according to the WHO classification 2008 between Japanese and German patients with refractory anemia according to the FAB classification in myelodysplastic syndromes

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ABSTRACT

We reported the different clinical features between Japanese and German refractory anemia (RA) patients in FAB classification. We re-analyzed the clinical features by WHO classification revised in 2008. The frequencies of refractory cytopenia with unilineage dysplasia (RCUD) and myelodysplastic syndrome-unclassified (MDS-U) with pancytopenia in Japanese patients were higher than in German patients ($p < 0.001$). Refractory cytopenia with multilineage dysplasia patients showed the most unfavorable prognosis in both countries. The higher frequencies of MDS-U with pancytopenia and RCUD in Japanese patients may influence the different clinical characteristics between Japanese and German FAB-RA patients.

1. Introduction

Myelodysplastic syndromes (MDS) are acquired clonal stem cell disorders characterized by ineffective hematopoiesis with myelodysplasia [1] and are associated with a high risk of progression to acute leukemias [2]. MDS are very heterogeneous in terms of their morphology, clinical features, and survival [3]. There are several reports indicating possible differences in clinical features between Western MDS types and Eastern MDS types [4–9]. The median age of MDS patients in Korea and Thailand were reported to be 57 [8] and 56 [7], respectively. On the other hand, large MDS studies from Western countries showed a median or mean age of 68–73 years [10–13]. We have reported that the clinical features of refractory anemia with excess of blasts (RAEB) or RAEB in transformation (RAEB-t) according to the French–American–British (FAB) classification [14] seemed to be similar between Japanese and Western patients [15]. However, previous reports [5,15] indicated that Japanese MDS patients have a lower frequency of refractory anemia with ringed sideroblasts (RARS) according to the FAB classification and a higher frequency of refractory anemia according to the FAB classification (FAB-RA) than the Western International Prognostic Scoring System (IPSS) study [10], and we reported that the clinical and laboratory features of Japanese FAB-RA patients apparently differ from those of German patients after a precise morphologic consensus (FAB classification: concordance rate, 98.4%; κ, 0.94; $p < 0.001$; prior World Health Organization (WHO) classification (WHO classification 2001) [16]: concordance rate, 83.8%; κ, 0.73; $p < 0.001$) [17]. That was the first comparison report between Western and Eastern FAB-RA patients after confirming morphological consensus. Japanese FAB-RA patients were younger, showed more severe cytopenia(s), a lower frequency of abnormal karyotypes, a lower frequency of MDS with isolated del(5q) (5q-syndrome), and a more favorable prognosis in terms of the overall survival (OS) and leukemia free survival (LFS) in our previous study.

MDS subtypes in the WHO classification 2001 [16] was revised in 2008 (WHO classification 2008) [18]. Refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT) were combined into refractory cytopenia with unilineage dysplasia

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(RCUD) in the WHO classification 2008. The diagnosis of MDS-unclassified (MDS-U) according to the WHO classification 2008 can be made in the following instances:

1. Patients with the findings of RCUD or refractory cytopenia with multilineage dysplasia (RCMD) but with 1% blasts in the peripheral blood (PB) (PB blasts type).
2. Cases of RCUD which are associated with pancytopenia (RCUD/pancytopenia type).
3. Patients with cytopenia(s) with 1% or fewer blasts in the PB and fewer than 5% in the bone marrow (BM), unequivocal dysplasia in <10% of the cells in one or more myeloid lineages, and who have cytogenetic abnormalities (cytogenetic abnormalities type).

MDS-U (PB blasts type) is classified as RAEB according to the FAB classification because of 1% blasts in the PB. MDS-U (cytogenetic abnormalities type) is not diagnosed as MDS according to the FAB classification because of unequivocal dysplasia. Thus, FAB-RA patients are classified as RCUD, RCMD, MDS with isolated del(5q) (5q- syndrome) or MDS-U (RCUD/pancytopenia type) according to the WHO classification 2008. In the present study, we re-analyzed in detail the clinical features of Japanese and German FAB-RA patients by using revised MDS subtypes in the WHO classification 2008.

2. Patients and methods

The dataset of consecutive patients with primary FAB-RA of our previous study [17] (total 728 consecutive patients: Japan, 131 cases; Germany, 597 cases) were used for the present retrospective analysis. Japanese patients of this dataset were diagnosed at the Saitama Medical University Hospital, Nagasaki University Hospital or affiliated hospitals between April 1976 and January 1997. German patients were diagnosed at the Department of Hematology, Oncology and Clinical Immunology of the Heinrich-Heine University between January 1973 and December 2002. Patients who had previously been treated with anti-neoplastic drugs or ionizing radiation were excluded from the study. Patients without the available necessary data for the WHO classification 2008 were excluded from the present study. Cytogenetic analyses were performed with a trypsin-Giemsa banding technique on BM cells from aspirates. Ordinarily 20–30 metaphases were examined. Cytogenetic aberrations were grouped according to the IPSS publication [10]. Thresholds for cytopenia(s) were defined as those of the IPSS (hemoglobin (Hb) <10.0 g/dL, absolute neutrophil count (ANC) $<1.8 \times 10^9$/L, and platelet $<100 \times 10^9$/L). Criteria for dysplasia were defined as those of a previous German report [19]. Hypoplastic BM was defined as <30% cellular in patients <60 years old, or <20% cellular in patients ≥60 years old [20]. If hypoplastic BM and certain dysplasia more than 10% in one or more of major myeloid cell lines were present, a diagnosis of hypoplastic MDS was made. Patients were reclassified according to the definition of WHO classification 2008 for MDS subtyping by using PB and BM findings, morphologic findings, and cytogenetic findings of the previous dataset [17]. Comparisons of the clinical features at the time of diagnosis and OS and LFS were analyzed by using the dataset of our previous study [17]. OS was measured from the date of diagnosis until death due to any cause, the date of stem cell transplantation, or until the last patient contact. LFS was measured from the date of diagnosis of acute leukemia. This study was approved by the Institutional Review Board of Saitama International Medical Center, Saitama Medical University, Saitama, Japan.

2.1. Statistical methods

The chi-square test and the nonparametric Mann–Whitney test were used to compare the proportions of patients and continuous data, respectively. The Kaplan–Meier method was used to generate the estimate of cumulative probabilities of OS and LFS. The difference in the cumulative probabilities within subcategories of patients was compared using a two-sided log-rank test. A two-sided p value of <0.05 was considered to be statistically significant. All statistical analyses were performed with the use of StatView (version 5.0, SAS Institute, Cary, NC).

3. Results

3.1. Comparison of frequencies of subtypes according to the WHO classification 2008 between Japanese and German FAB-RA patients

A total of 295 patients (Japan, 102 cases; Germany, 193 cases) could be classified according to the WHO classification 2008. A total of 433 patients (Japan, 29 cases; Germany, 404 cases) could not be classified according to the WHO classification 2008 due to a deficit of either cytogenetic data or adequate peripheral blood data, and 427 patients presented without available cytogenetic findings (Japan, 29 cases; Germany, 398 cases). There were 6 patients (Germany, 6 cases) without any data of peripheral blood.

MDS-U (PB blasts type) is classified as RAEB according to the FAB classification. MDS-U (cytogenetic abnormalities type) is not diagnosed as MDS according to the FAB classification due to unequivocal dysplasia. Therefore, patients with MDS-U (PB blasts type) or with MDS-U (cytogenetic abnormalities type) were not included in the previous dataset. Because the previous dataset used in the present study was that of FAB-RA patients, dysplasia existed in at least one lineage and the frequency of blasts in PB was <1% in all patients. Therefore, all MDS-U patients in the present study were diagnosed as RCUD/pancytopenia type. Most Japanese FAB-RA patients were classified as RCUD, RCMD, or MDS-U (RCUD/pancytopenia type) according to the WHO classification 2008 (Table 1A). Most German FAB-RA patients were classified as RCUD, RCMD, or 5q- syndrome (Table 1B). The frequency of RCUD in Japanese FAB-RA patients (45%) was significantly higher than that in German FAB-RA patients (19%) ($p < 0.001$). The frequency of patients with bicytopenia in Japanese RCUD patients was 59%, but that in the German RCUD patients was only 19%. Among 46 Japanese RCUD patients, number of patients with single cytopenia was 17 cases (37%) including 2 RA, 4 RN and 11 RT cases. Among 37 German RCUD patients, number of patients with single cytopenia was 22 cases (59%) including 7 RA, 11 RN and 4 RT cases. Frequency of RT was 2% of German FAB-RA patients. The frequency of RT of Japanese FAB-RA patients (11%) was higher than that of German FAB-RA patients. The frequency of MDS-U in Japanese FAB-RA patients (29%) was significantly higher than that in German FAB-RA patients (3%) ($p < 0.001$). The frequency of RCMD in Japanese FAB-RA patients (25%) was significantly lower than in German FAB-RA patients (58%) ($p < 0.001$). The frequency of 5q- syndrome in Japanese FAB-RA patients (3%) was significantly lower than in German FAB-RA patients (20%) ($p < 0.001$) (Table 1C).

3.2. Comparison of clinical and laboratory features at the time of diagnosis between Japanese and German patients could be classified according to the WHO classification 2008

The age of patients in RCUD, MDS-U and RCMD subtypes did not differ between the two countries. The MDS-U (RCUD/pancytopenia type) subtype was younger than other subgroups in Japanese patients. The gender ratios in the RCUD

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