complete remission of ATL.[19] Similar observations have been rarely reported in other aggressive mature lymphoid neoplasms,[21] suggesting the unique susceptibility of ATL to graft-versus-host reactions. Recently, a combined analysis of 2 prospective studies including 29 ATL patients in total undergoing allogeneic HCT suggested that development of mild acute GVHD favorably affected overall survival and progression-free survival.[22] However, the impact of GVHD on the outcome of allogeneic HCT in ATL needs to be verified in a much larger cohort. We previously conducted a nationwide retrospective study to evaluate the current results of allogeneic HCT for ATL, and we confirmed that a substantial proportion of patients with ATL can enjoy long-term, disease-free survival after transplantation: the overall survival rate at 3 years among patients who received transplants in complete remission and not in complete remission was 51% and 26%, respectively.[23] Using the same cohort, we further evaluated the effects of acute and chronic GVHD on long-term outcomes of allografted patients with ATL.

Methods

Collection of data

Data on 417 patients with acute or lymphoma type ATL who had undergone allogeneic bone marrow, peripheral blood, or cord blood transplantation between January 1, 1996, and December 31, 2005, were collected through the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN), the 3 largest HCT registries in our country; their roles were detailed previously.[23] The patients were included from 102 transplant centers; the data were updated as of December 2008. The study was approved by the data management committees of JSHCT, JMDP, and JCBBN, as well as by the institutional review boards of Kyoto University Graduate School of Medicine, where this study was organized.

Inclusion and exclusion criteria

Patients were included in the analysis if the following data were available: age at transplantation, sex of the recipient, donor type, stem cell source, agents used in the conditioning regimen and GVHD prophylaxis, the maximum grade and day of occurrence of acute GVHD, and the day of neutrophil recovery. Acute GVHD was reported according to the traditional criteria,[24] except that 1 patient was considered to have late-onset acute GVHD at day 133; neutrophil recovery was considered to have occurred when an absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation. Patients who missed any of these data ($n = 37$), who had a history of prior autologous or allogeneic HCT ($n = 8$), who had received an ex vivo T cell–depleted graft ($n = 1$), who experienced primary or secondary graft failure ($n = 24$) were excluded from the analysis. Because the association between the occurrence of acute GVHD and disease-associated mortality was difficult to evaluate in the event of early toxic death, patients who died within 30 days of transplantation ($n = 53$) also were excluded from the study. Among these 53 patients, 22 were evaluable for acute GVHD: grade 0 in 17 patients, grade 1-2 in 3 patients, and grade 3-4 in 2 patients. Two physicians (J.K. and T.I.) independently reviewed the quality of collected data, and 294 patients in total (158 males and 136 females), with a median age of 51 years (range, 18-79 years), were found to meet these criteria and included in the study: 163 patients from JSHCT, 82 patients from JMDP, and 49 patients from JCBBN. No overlapping cases were identified. Of these 294 patients, the effects of chronic GVHD, reported and graded according to using traditional criteria,[25] were considered evaluable for the 183 patients who survived at least 100 days after transplantation with complete information on the type and the day of occurrence of chronic GVHD.

End points

The primary end point of the study was the effect of acute GVHD on overall survival, defined as the period from the date of transplantation until the date of death from any cause or the last follow-up. The secondary end points of the study included the impact of acute GVHD on disease-associated and treatment-related mortality, and the impact of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality. Reported causes of death were reviewed and categorized into disease-associated or treatment-associated deaths. Disease-associated deaths were defined as deaths from relapse or progression of ATL, whereas treatment-related deaths were defined as any death other than disease-associated deaths.

Statistical analysis

The probability of overall survival was estimated by the Kaplan-Meier method. Treatment-related and disease-associated mortality were estimated with the use of cumulative incidence curves to accommodate the following competing events[26]: disease-associated death for treatment-related mortality and treatment-related deaths for disease-associated mortality. Data on patients who were alive at the time of last follow-up were censored. Semi-landmark plots were used to illustrate the effects of GVHD on overall survival and cumulative incidence of disease-associated and treatment-related deaths. For patients with acute or chronic GVHD, the probability of overall survival and the cumulative incidences of disease-associated and treatment-related deaths were plotted as a function of time from the onset of acute or chronic GVHD. Day 24.5, the median day of onset for acute GVHD, was termed as the landmark day in patients without acute GVHD. In the case of patients without chronic GVHD, day 116, the median day of onset for chronic GVHD, was termed as the landmark day.

Univariate and multivariate Cox proportional hazards regression models were used to evaluate variables potentially affecting overall survival, whereas the Fine and Gray proportional subdistribution hazards models were used to evaluate variables potentially affecting disease-associated and treatment-related mortality.[27] In these regression models, the occurrence of acute and chronic GVHD was treated as a time-varying covariate.[28] In the analysis of acute GVHD, patients were assigned to the "no acute GVHD group" at the time of transplantation and then transferred to the "grade 1-2 acute GVHD group" or to the "grade 3-4 acute GVHD group" at the onset of the maximum grade of acute GVHD. In the analysis of chronic GVHD, patients were assigned to the "no chronic GVHD group" at the time of transplantation and then transferred to the "limited chronic GVHD group" or to the "extensive chronic GVHD group" at the onset of the maximum grade of chronic GVHD. The variables considered were the age group of the recipient (≤ 50 years or > 50 years at transplantation), sex of the recipient (female or male), disease status before transplantation (complete remission, disease status other than complete remission, or unknown), intensity of conditioning regimen (myeloablative, reduced intensity, or unclassifiable), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other), type of donor (HLA-matched related donor, HLA-mismatched related donor, unrelated donor for bone marrow, or unrelated cord blood), time from diagnosis to transplantation (within 6 months, > 6 months, or unknown), and year of transplantation (1995-2002 or 2003-2005). We classified the intensity of conditioning regimen as myeloablative or reduced intensity based on the working definition by Center for International Blood and Marrow Transplant Research if data on dosage of agents and total-body irradiation (TBI) used in the conditioning regimen were available.[29] For 110 patients for whom such information was not fully available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by treating clinicians. The cutoff points for year of transplantation were chosen such that we could make optimal use of the data with a proviso that the smaller group contained at least 30% of patients. In the analysis of the effect of chronic GVHD, the prior history of grade 2-4 acute GVHD also was added to the multivariate models. We also assessed the interaction between acute GVHD and the intensity of conditioning regimen in the multivariate models. Only factors with a P value of less than .10 in univariate analysis were included in the multivariate models. In addition, the heterogeneities of the effects of grade 1-2 or grade 3-4 acute GVHD on overall survival according to background transplant characteristics were evaluated by the forest plots stratified by variables included in the regression analyses. Furthermore, landmark analysis treating the development of acute GVHD as a time-fixed covariate was performed to confirm

Table 1. Characteristics of patients and transplants

Variable	No. of patients, n = 294 (%)
Age group at transplant, y	
≤ 30	7 (2)
> 30-40	30 (10)
> 40-50	109 (37)
> 50-60	123 (42)
> 60	25 (9)
Sex	
Male	158 (54)
Female	136 (46)
Disease status	
Complete remission	99 (34)
Not in complete remission	178 (61)
Unknown	17 (6)
Conditioning regimen	
Myeloablative	102 (34)
Reduced intensity	128 (44)
Unclassifiable	64 (22)
GVHD prophylaxis*	
Cyclosporine-based	195 (66)
Tacrolimus-based	94 (32)
Other	5 (2)
Source of stem cells	
Bone marrow	132 (45)
Peripheral blood	111 (38)
Bone marrow + peripheral blood	2 (1)
Cord blood	49 (17)
Type of donor†	
HLA-matched related	132 (45)
HLA-mismatched related	31 (11)
Unrelated, bone marrow	82 (28)
Unrelated, cord blood	49 (17)
Time from diagnosis to transplant	
≤ 6 mo	141 (48)
> 6 mo	141 (48)
Uncertain/missing	12 (4)
Year of transplant	
1995-1999	22 (7)
2000-2002	91 (31)
2003-2005	181 (62)
Follow-up of survivors	
Median time, mo (range)	42.8 (1.5-102.3)

Data are numbers (%) unless specified otherwise.

*Cyclosporine-based indicates cyclosporine with or without other agents; tacrolimus-based indicates tacrolimus with or without other agents.

†HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, B, and DR antigens.

the results of analyses treating the occurrence of acute GVHD as a time-varying covariate; the landmark day was set at day 68 after transplantation, the date until when more than 95% of patients developed acute GVHD.

Results are expressed as hazard ratios (HRs) and their 95% confidence intervals (CI). All tests were 2-sided, and a *P* value of less than .05 was considered to indicate statistical significance. All statistical analyses were performed with STATA Version 11 software (StataCorp).

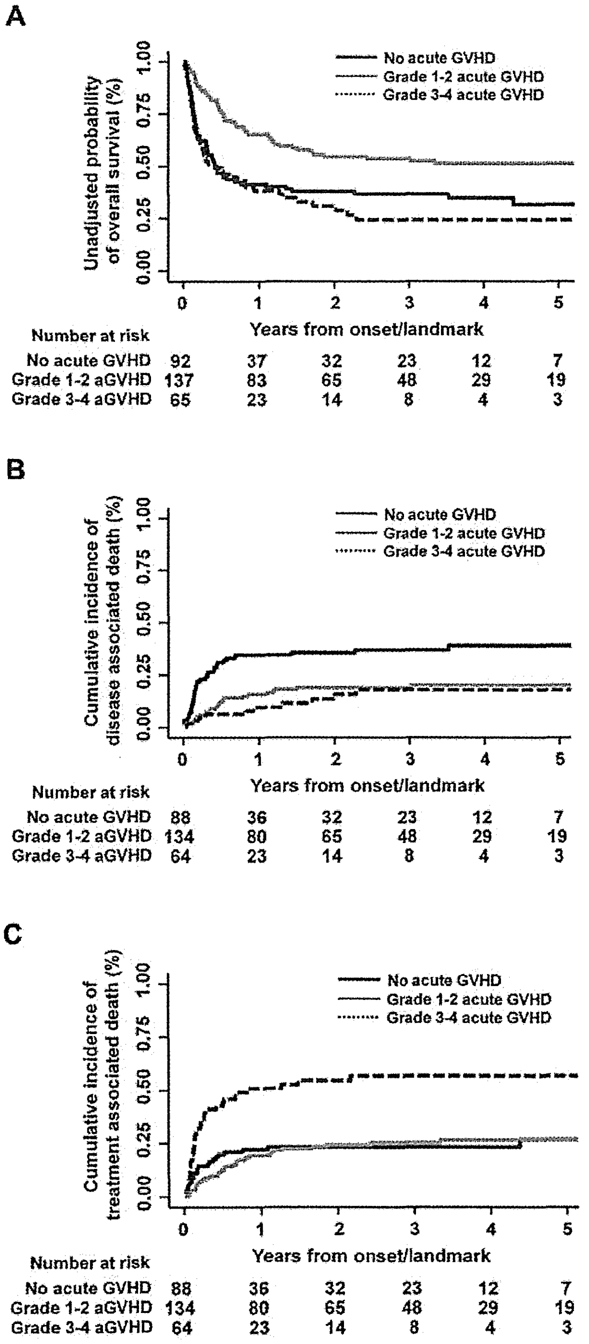
Results

Characteristics of patients and transplants

Characteristics of the patients and transplants are shown in Table 1. Most of the patients received transplants at the age of 41 to 60 years (median, 51 years). The disease status at transplantation was mainly defined as other than complete remission. The intensity of conditioning regimen was classified as myeloablative in 102 (35%) patients and reduced intensity in 128 (44%) patients; the remaining 64 (22%) patients were reported to receive cyclophosphamide plus TBI in 16 patients; busulfan plus cyclophosphamide in 15 patients; busulfan plus melphalan in 1 patient; purine analog-containing regimen in 6 patients; and other TBI-based regimens in 26 patients, although the intensity of these regimens was considered unclassifiable because of lack of dosage information. Cyclosporine-based prophylaxis against GVHD was used in more than half of patients. Patients underwent transplantation using HLA-matched related donor in 132 patients (45%), HLA-mismatched related donor in 31 patients (11%), unrelated bone marrow donor in 82 patients (28%), and unrelated cord blood unit in 49 patients (17%). Half of the patients received transplants within 6 months of diagnosis. The median time of follow-up among the survivors was 42.8 months (range, 1.5-102.3 months).

Effects of acute GVHD on overall survival

The median onset day of acute GVHD of any grade after transplantation was 24.5 (range, 5-133). Acute GVHD of grades 1-4, 2-4, and 3-4 occurred in 202 patients (69%), 150 patients (51%), and 65 patients (22%), respectively. The effect of acute GVHD on overall survival was evaluated using semi-landmark plots with reference to the following 3 categories: no acute GVHD, grade 1-2 acute GVHD, and grade 3-4 acute GVHD (Figure 1A). The impact of grade 1-2 or grade 3-4 acute GVHD on overall survival also was evaluated by forest plots stratified by background characteristics of patients and transplants (Figure 2). These analyses revealed that development of grade 1-2 acute GVHD was consistently associated with higher overall survival compared with the absence of acute GVHD, whereas occurrence of grade 3-4 acute GVHD was consistently associated with lower overall survival, except that adverse impact of grade 3-4 acute GVHD was not observed in the subgroups of patients who received transplants from an HLA-matched related or HLA-mismatched related donor. Multivariate analysis treating an occurrence of acute GVHD as a time-dependent covariate also confirmed the positive impact of grade 1-2 acute GVHD (HR, 0.65; 95% CI, 0.45-0.93; *P* = .018) and the adverse impact of grade 3-4 acute GVHD on overall survival (HR, 1.64; 95% CI, 1.10-2.42; *P* = .014; Table 2). Patients who received reduced intensity conditioning and myeloablative conditioning had similar rates of overall survival by both univariate (HR of reduced intensity vs myeloablative transplant, 1.19; 95% CI, 0.85-1.68; *P* = .318) and multivariate analysis (HR, 0.95; 95% CI, 0.61-1.47; *P* = .814). There was no interaction effect between conditioning intensity and grade 1-2 (*P* = .704) or grade 3-4 acute GVHD (*P* = .891) on overall survival. The effect of each grade of acute GVHD on overall survival was additionally evaluated. It showed that only grade 2 acute GVHD was associated with superior overall survival, whereas only grade 4 acute GVHD was associated with inferior survival (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). In the landmark analysis treating an occurrence of acute GVHD as a time-fix covariate, consistent results were obtained for patients who survived at least 68 days (landmark day), although the adverse impact of grade 3-4 acute GVHD on overall survival became no longer significant (supplemental Table 2).



A

Number at risk						
No acute GVHD	92	37	32	23	12	7
Grade 1-2 aGVHD	137	83	65	48	29	19
Grade 3-4 aGVHD	65	23	14	8	4	3

B

Number at risk						
No acute GVHD	88	36	32	23	12	7
Grade 1-2 aGVHD	134	80	65	48	29	19
Grade 3-4 aGVHD	64	23	14	8	4	3

C

Number at risk						
No acute GVHD	88	36	32	23	12	7
Grade 1-2 aGVHD	134	80	65	48	29	19
Grade 3-4 aGVHD	64	23	14	8	4	3

Figure 1. Semi-landmark plots for effects of acute GVHD. Semi-landmark plots illustrating the effects of acute GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

Effects of acute GVHD on disease-associated and treatment-related mortality

We next evaluated the effects of acute GVHD on disease-associated and treatment-related mortality (Figure 1B-C). Disease-associated mortality was defined as cumulative incidence of death directly attributable to relapse or progression of ATL, whereas treatment-related mortality was calculated as cumulative incidence of any death not included in disease-associated deaths. Multivariate analysis revealed that disease-associated mortality was lower in the presence of grade 1-2 and grade 3-4 acute GVHD compared with the absence of acute GVHD (grade 1-2 acute GVHD: HR, 0.54; 95% CI, 0.32-0.92; $P = .023$ and grade 3-4 acute GVHD: HR, 0.44; 95% CI, 0.22-0.90; $P = .024$; Table 2), and each grade of acute GVHD showed consistent inverse association with disease-associated mortality (supplemental Table 1). Although the risk of treatment-related mortality was not higher in the presence of grade 1-2 acute GVHD, development of grade 3-4 acute GVHD was significantly associated with higher treatment-related mortality compared with the absence of acute GVHD (HR, 3.50; 95% CI, 2.01-6.11; $P < .001$; Table 2). Patients undergoing reduced intensity transplantation and those undergoing myeloablative transplantation had similar risks of disease-associated death (HR, 0.99; 95% CI, 0.46-2.13; $P = .975$) and treatment-related death (HR, 0.98; 95% CI, 0.60-1.59; $P = .928$) by multivariate analysis. There was no interaction effect between conditioning intensity and grade 1-2 or grade 3-4 acute GVHD on disease-associated mortality and treatment-related mortality. Of 95 patients who experienced treatment-related deaths, 27 patients succumbed to infectious complications: bacterial in 13 patients, viral in 7 patients (including 3 cases of cytomegalovirus disease), viral and bacterial in 1 patient, fungal in 5 patients, and no specific organism reported in 1 patient. The proportions of patients who died of infectious complication among those without acute GVHD ($n = 92$), those with grade 1-2 ($n = 137$), and those with grade 3-4 acute GVHD ($n = 65$) were 4%, 9%, and 17%, respectively (supplemental Table 3). By multivariate analysis, development of grade 3-4 acute GVHD was significantly associated with higher risk of death related to infection (HR, 4.74; 95% CI, 1.51-14.8; $P = .008$), whereas the adverse influence on the infection-related deaths was less evident in the presence of grade 1-2 acute GVHD (HR, 2.17; 95% CI, 0.72-6.56; $P = .169$).

Effects of chronic GVHD on overall survival and mortality

Chronic GVHD was evaluated in 183 patients who survived at least 100 days after transplantation. The median day of chronic GVHD occurrence after transplantation was 116 (range, 100-146 days). Limited and extensive chronic GVHD occurred in 29 (16%) and 63 patients (34%), respectively. Semi-landmark plots were constructed to illustrate the effects of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality with reference to the following subgroups: no chronic GVHD, limited chronic GVHD, and extensive chronic GVHD (Figure 3). In multivariate analysis treating an occurrence of chronic GVHD as a time-dependent covariate, neither overall survival nor disease-associated mortality was significantly associated with severity of chronic GVHD, whereas treatment-related mortality was higher in the presence of extensive chronic GVHD (HR, 2.75; 95% CI, 1.34-5.63; $P = .006$) compared with the absence of chronic GVHD (Table 3). The proportions of patients who died of infectious complication among those without chronic GVHD ($n = 91$), those with limited chronic GVHD ($n = 29$), and those with extensive chronic GVHD ($n = 63$) were 7%, 10%, and 8%, respectively. In multivariate analysis, no statistically significant association was found between infection-related death and the occurrence of either limited ($P = .289$) or extensive GVHD ($P = .836$).

Discussion

To our knowledge, this is the largest retrospective study to analyze the impact of acute and chronic GVHD on clinical

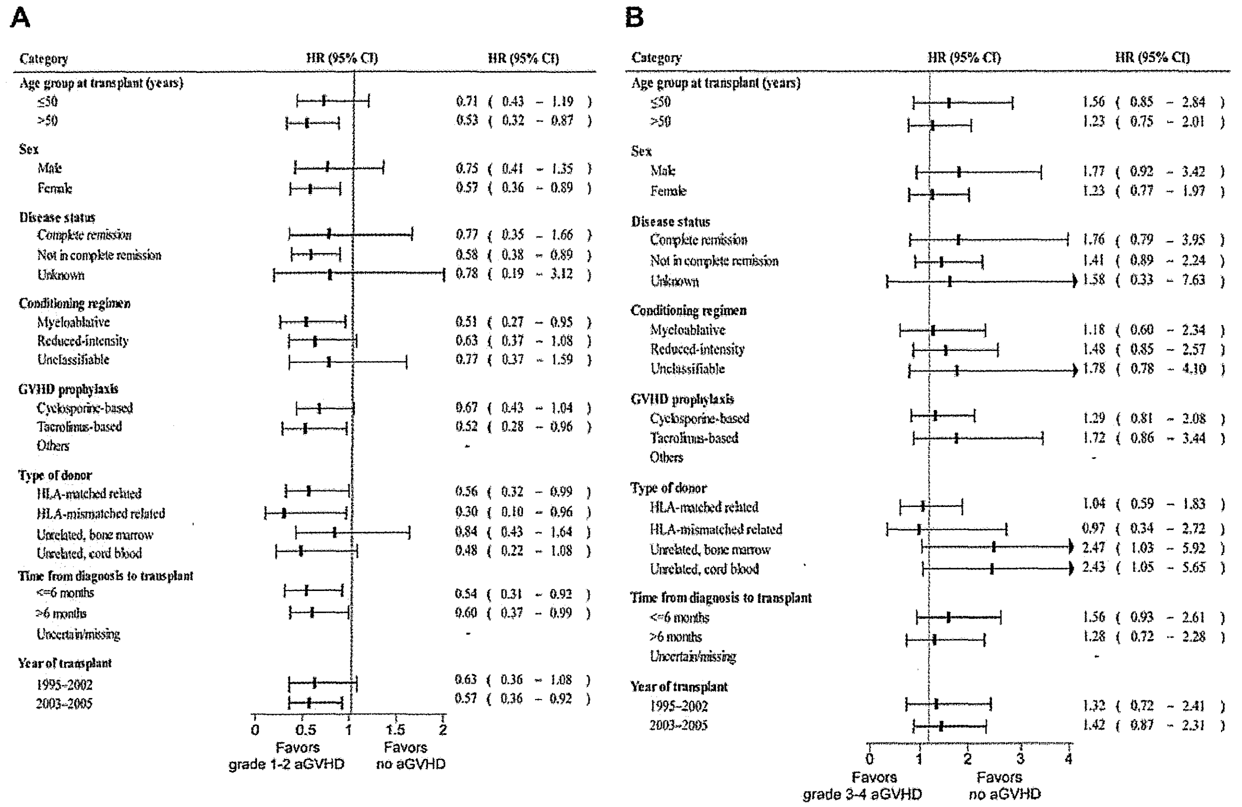


Figure 2. Impact of the grade of acute GVHD on overall survival in each stratified category. Effects of grade 1-2 (A) and grade 3-4 acute GVHD (B) on overall survival are shown as forest plots. Square boxes on lines indicate hazard ratios compared with "no acute GVHD group," and horizontal lines represent the corresponding 95% CI. Abbreviations used are the same as described in the footnotes to Tables 1 and 2.

outcomes including overall survival, disease-associated mortality, and treatment-related mortality after allogeneic HCT for ATL. In the present study, the occurrence of both grade 1-2 and grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD. However, positive effect of GVHD on reduced disease-associated mortality was counterbalanced by increased treatment-related mortality among patients who developed severe acute GVHD, and an overall beneficial effect on survival was observed only with the development of mild-to-moderate acute GVHD. In contrast to acute GVHD, no beneficial effect was observed in association with the development of chronic GVHD, although the point estimate of the HR comparing limited chronic GVHD versus the absence of chronic GVHD

Table 2. Effect of acute GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	HR (95% CI)	*P*	HR (95% CI)	*P*
Overall survival*				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.60 (0.42-0.85)	.004	0.65 (0.45-0.93)	.018
Grade 3 or 4 acute GVHD vs no acute GVHD	1.38 (0.94-2.01)	.099	1.64 (1.10-2.42)	.014
Disease-associated mortality†				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.47 (0.28-0.79)	.005	0.54 (0.32-0.92)	.023
Grade 3 or 4 acute GVHD vs no acute GVHD	0.41 (0.21-0.81)	.010	0.44 (0.22-0.90)	.024
Treatment-related mortality‡				
Grade 1 or 2 acute GVHD vs no acute GVHD	1.13 (0.67-1.89)	.649	1.22 (0.72-2.07)	.461
Grade 3 or 4 acute GVHD vs no acute GVHD	3.34 (1.94-5.74)	< .001	3.50 (2.01-6.11)	< .001

*Other significant variables were sex of recipient, female (reference, 1.00) and male (HR, 1.70; 95% CI, 1.24-2.32; *P* = .001); achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.05; 95% CI, 1.44-2.92; *P* < .001), and status not known (HR, 2.21; 95% CI, 1.15-4.22; *P* = .017); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 1.71; 95% CI, 1.04-2.84; *P* = .036), unrelated donor of bone marrow (HR, 1.39; 95% CI, 0.94-2.06; *P* = .096), and unrelated cord blood (HR, 1.86; 95% CI, 1.22-2.83; *P* = .004).

†Other significant variables were achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.98; 95% CI, 1.62-5.47; *P* < .001), and status not known (HR, 0.96; 95% CI, 0.21-4.49; *P* = .963); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 2.14; 95% CI, 1.00-4.55; *P* = .049), unrelated donor of bone marrow (HR, 1.45; 95% CI, 0.81-2.61; *P* = .214), and unrelated cord blood (HR, 1.25; 95% CI, 0.63-2.49; *P* = .517).

‡Another significant variable was achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 1.17; 95% CI, 0.74-1.84; *P* = .498) and status not known (HR, 2.31; 95% CI, 1.04-5.15; *P* = .040).

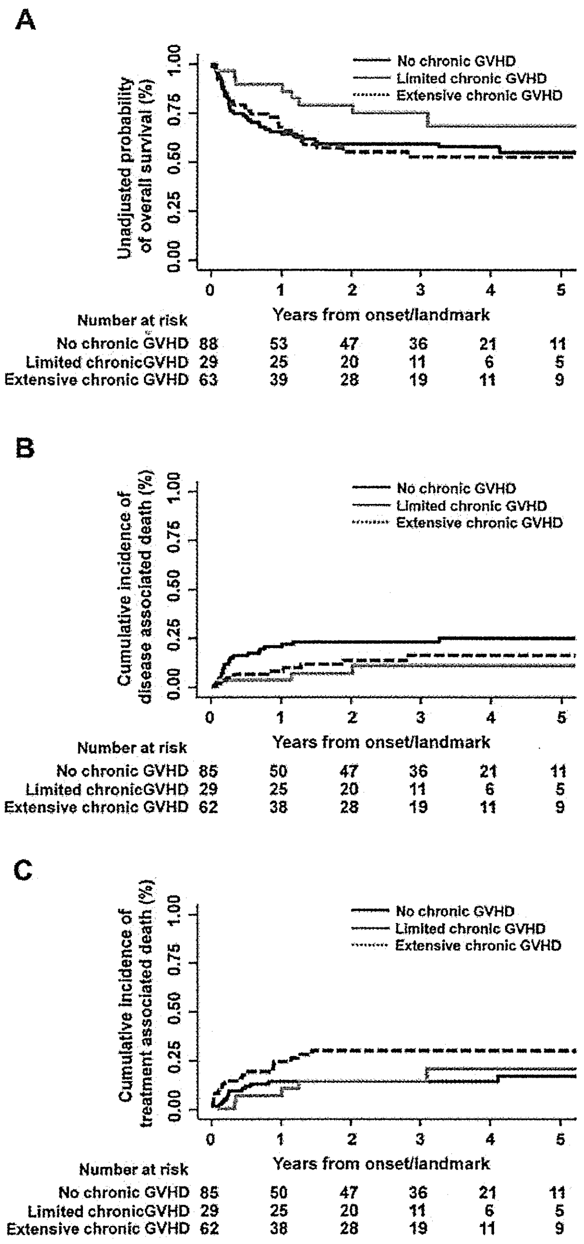


Figure 3. Semi-landmark plots for impact of chronic GVHD. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

suggested the trend toward a reduced risk of disease-associated deaths in the limited chronic GVHD group.

Our present findings are in contrast to the previous reports showing the beneficial effects of chronic GVHD rather than acute GVHD on the prevention of disease recurrence after allogeneic HCT. It is less likely that the particular characteristics of chronic GVHD in patients with ATL biased the results, because the incidence rate and median onset day of chronic GVHD in our cohort were similar to those reported in previous studies evaluating the incidence of chronic GVHD among Japanese patients, most of whom had received allogeneic HCT for myeloid neoplasms or acute lymphoblastic leukemia.[30-32] Conceivably, the rapid tempo of disease recurrence of ATL might be such that chronic GVHD is less potent in terms of harnessing clinically relevant graft-versus-leukemia responses compared with acute GVHD. However, the results of our analysis regarding the effect of chronic GVHD should be interpreted with caution because the number of patients evaluable for chronic GVHD was relatively small in our study for providing sufficient statistical power. The effect of chronic GVHD on outcomes after HCT for ATL should be further explored in a larger cohort.

The occurrence of GVHD has been shown to exert a potent graft-versus-leukemia effect in terms of reducing relapse incidence in acute leukemia or chronic myeloid leukemia.[33,34] In contrast, multiple studies have documented a correlation between GVHD in its acute or chronic form and treatment-related mortality. In a study of patients undergoing HLA-identical sibling HCT for chronic myeloid leukemia, the overall beneficial effect on long-term survival was demonstrated only in a group of patients who developed grade 1 acute GVHD or limited chronic GVHD.[33] In another study of HLA-identical sibling HCT for leukemia using cyclosporine and methotrexate as GVHD prophylaxis, a benefit of mild GVHD was only seen in high-risk patients but not in standard-risk patients. Therefore, the therapeutic window between decreased relapse incidence and increased transplant-related mortality in association with the development of GVHD has been considered to be very narrow.[34]

With regard to the effectiveness of allogeneic HCT for ATL, it is also of note here that posttransplant eradication of ATL cells can be achieved without the use of high-dose chemoradiotherapy: patients who received a transplant with reduced intensity conditioning had survival outcomes similar to those who received a transplant with myeloablative conditioning in our study. Intriguingly, several small cohort studies exhibited that abrupt discontinuation of immunosuppressive agents resulted in disappearance or reduction in the tumor burden in allografted patients with ATL. In some cases, remission of ATL was observed along with the development of GVHD.[19,20,22] Taken together with the findings of this study, it is suggested that ATL is particularly susceptible to immune modulation following allogeneic HCT. To clarify the presence of such "graft-versus-ATL" effect, further investigations are needed to assess the efficacy of donor lymphocyte infusion or withdrawal of immunosuppressive agents on relapse after transplantation.

Of the HTLV-I gene products, Tax is a dominant target of HTLV-I–specific cytotoxic T lymphocytes. The vigorous Tax-specific cytotoxic T-cell responses were demonstrated in recipients who obtained complete remission after allogeneic HCT for ATL, suggesting that "graft-versus-HTLV-I" responses might contribute to the eradication of ATL cells.[35,36] However, Tax is generally undetectable or present in very low levels in primary ATL cells.[37,38] In addition, small amounts of HTLV-I provirus can be detected in peripheral blood of recipients who attained long-term remission of ATL, even after HCT from HTLV-I–negative donors.[39,40] These findings suggest that "graft-versus-ATL" effect can be harnessed without complete elimination of HTLV-I. It is also important to note that allogeneic HCT is emerging as an effective treatment option for other mature T-cell neoplasms not related to HTLV-I, such as mycosis fungoides/Sézary syndrome and various types of aggressive peripheral T-cell lymphomas.[41,42] These observations raised the possibility that the common targets for alloimmune responses might exist across a spectrum of malignant T-cell neoplasms, including ATL. The minor histocompatibility antigens or tumor-specific antigens can be other targets of alloimmune anti-ATL effect.[43-45] Therefore, the elucidation of the mechanism underlying an immunologic eradication of primary ATL cells may

Table 3. Effect of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
Overall survival*				
Limited chronic GVHD vs no chronic GVHD	0.71 (0.34-1.47)	.353	0.72 (0.35-1.50)	.385
Extensive chronic GVHD vs no chronic GVHD	1.45 (0.90-2.35)	.131	1.40 (0.86-2.30)	.176
Disease-associated mortality†				
Limited chronic GVHD vs no chronic GVHD	0.45 (0.14-1.46)	.183	0.45 (0.14-1.44)	.178
Extensive chronic GVHD vs no chronic GVHD	0.81 (0.39-1.67)	.563	0.80 (0.39-1.64)	.536
Treatment-related mortality‡				
Limited chronic GVHD vs no chronic GVHD	1.59 (0.64-3.95)	.316	1.56 (0.63-3.87)	.342
Extensive chronic GVHD vs no chronic GVHD	2.85 (1.41-5.77)	.004	2.75 (1.34-5.63)	.006

*There was no significant variable.
†There was no significant variable.
‡There was no other significant variable.

lead to a new strategy for improving outcomes of allogeneic HCT not only for ATL but also for other intractable T-cell neoplasms.

This study has several limitations. First, acute GVHD might be intentionally induced for some patients considered at high risk of relapse by treating clinicians. Second, the information on the day when each grade of GVHD occurred was not available. Therefore, we treated the development of acute and chronic GVHD in their worst severity as a time-varying covariate. To validate the results, we also performed the landmark analysis and obtained consistent results. Third, the relatively small number of patients with chronic GVHD might mask or bias the effect of chronic GVHD on outcomes. Last, the effect of multiple testing should be taken into account for the interpretation of the secondary end points.

In conclusion, the development of acute GVHD was associated with lower disease-associated mortality after allogeneic HCT for ATL compared with the absence of acute GVHD. However, improved survival can be expected only among a group of patients who developed mild-to-moderate acute GVHD because those who developed severe acute GVHD were at high risk of treatment-related mortality. New strategies that enhance the allogeneic anti-ATL effect without exacerbating GVHD are required to improve the outcomes of patients undergoing allogeneic HCT for ATL.

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The views expressed in this report are those of authors and do not indicate the views of the JSHCT, JMDP, or JCBBN.

This work is in memory of T.U., who died during the preparation of this manuscript.

Authorship

Contribution: T.I. and T.U. designed the research and organized the project; M. Hishizawa, J.K., T.I., and T.U. reviewed and analyzed data and wrote the paper; J.K., T.I., and K.M. performed statistical analysis; Y.A., R.S., and H.S. collected data from JSHCT; T.K. and Y. Morishima collected data from JMDP; T.N.-I., and S. Kato collected data from JCBBN; and A.U., S.T., T.E., Y. Moriuchi, R.T., F.K., Y. Miyazaki, M.M., K.N., M. Hara, M.T., S. Kai, and J.O. interpreted data and reviewed and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A list of other members who contributed data on allogeneic HSCT for ATL to JSHCT, JMDP, and JCBBN appears in the online supplemental Appendix.

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CASE REPORT

Successful treatment of a chronic-phase T-315I-mutated chronic myelogenous leukemia patient with a combination of imatinib and interferon-alfa

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Abstract The T315I BCR-ABL mutation in chronic myelogenous leukemia (CML) patients is responsible for up to 20% of all clinically observed resistance. This mutation confers resistance not only to imatinib, but also to second-generation BCR-ABL tyrosine kinases, such as nilotinib and dasatinib. A number of strategies have been implemented to overcome this resistance, but allogeneic stem cell transplantation remains the only established therapeutic option for a cure. A 61-year-old male was diagnosed with Philadelphia chromosome-positive chronic-phase CML in 2002. He was initially treated with imatinib and complete cytogenetic response (CCyR) was achieved 12 months later. However, after 18 months, a loss of CCyR was observed and a molecular study at 24 months revealed a T315I mutation of the BCR-ABL gene. At 30 months, imatinib/interferon-alfa (IFNα) combination therapy was initiated in an effort to overcome the resistance. Thirty months later, he re-achieved CCyR, and the T315I BCR-ABL mutation disappeared at 51 months. To our knowledge, this is the first case report showing the effectiveness of imatinib/IFNα combination therapy for CML patients bearing the T315I BCR-ABL mutation.

Keywords Chronic myelogenous leukemia · Imatinib · Interferon · T315I

Introduction

Chronic myelogenous leukemia (CML) is a clonal disease of the hematopoietic stem cell, which is characterized by an increased growth of predominantly myeloid cells in the bone marrow. The disease is associated with the Philadelphia chromosome, which arises by a reciprocal translocation between chromosomes 9 and 22 and harbors the BCR-ABL fusion oncogene [1]. Small molecules that specifically target the BCR-ABL gene product provide a successful treatment approach which can lead to a reduction in BCR-ABL transcripts below detectable levels. The drug imatinib, a rationally designed tyrosine kinase inhibitor (TKI), showed a superior response rate, improved progression-free survival, and overall survival, as compared with the previous standard therapy with IFNα [2–4].

Although high response rates are observed in patients who receive imatinib treatment, a small percentage of chronic-phase (CP) CML patients are refractory to the therapy [2]. Patients develop imatinib resistance via

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multiple mechanisms, with some being BCR-ABL dependent and others BCR-ABL independent. To overcome the failure of imatinib, multiple strategies are under investigation. These strategies include a dose escalation of imatinib and switching to second-generation TKIs. Nilotinib and dasatinib are currently approved for the treatment of patients with CML who have developed resistance or intolerance to imatinib [5, 6].

The development of a T315I BCR-ABL mutation (threonine to isoleucine mutation at amino acid 315) is of particular concern as it confers resistance to all available TKIs [7–10]. The only established salvage option for patients harboring the T315I BCR-ABL mutation is allogeneic hematopoietic stem cell transplantation (allo-HSCT) [11–13]. However, allo-HSCT can be performed only in eligible patients [14]. For patients who could not receive allo-HSCT, new agents with activity against the T315I BCR-ABL mutation, such as danusertib and omacetaxin, have been developed [15, 16]. However, they are still in the clinical trial stage and it will take years before these agents can be put into use. Hence, patients harboring the T315I BCR-ABL mutation, who are not eligible for allo-HSCT, require treatment with combinations of already approved drugs.

We report the successful treatment of a CML patient harboring the T315I BCR-ABL mutation with a combination of imatinib and IFNα.

Materials and methods

Total RNA extraction and cDNA synthesis

Total leukocytes in bone marrow and peripheral blood samples were isolated by centrifugation following red blood cell lysis and total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA). cDNA was synthesized using oligo-dT primers and Super Script III Reverse Transcriptase (Invitrogen).

TaqMan quantitative reverse transcriptase-polymerase chain reaction

Quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) for BCR-ABL transcript levels were performed using the LightCycler (Roche Diagnostics, Mannheim, Germany) and LightCycler TaqMan Master (Roche Diagnostics). Primers and TaqMan probe sequences published in the EAC network protocol were used for RQ-PCR [17]. The amount of the fusion gene in the original sample was calculated by means of a standard curve (created with the BCR-ABL fusion gene or the ABL gene cloned in plasmids) and expressed as the BCR-ABL/ABL ratio.

Direct sequencing of ABL kinase domain

A nested PCR sequencing approach was used for direct sequencing of the ABL kinase domain, with a first-round amplification of the BCR-ABL transcript followed by two separate PCR reactions. For the nested PCR, the primers were used as described previously [18, 19]. To screen for mutations, the PCR products were sequenced in both the directions with the following: ABL-1F (5′-ACAGGATCAACACTGCT TCTGA-3′); ABL-1R (5′-TGGCTGACGAGATCTGAGTG-3′); ABL-2F (5′-ATGGCCACTCAGATCTCGTC-3′); and ABL-2R (5′-GATACTGGATTCCTGGAACA-3′) using a BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI Prism 3100xl Genetic Analyzer (Applied Biosystems, CA, USA).

Quantitative T315I BCR-ABL mutational analysis by pyrosequencing

Quantitation of T315I BCR-ABL and un-mutated BCR-ABL transcript levels were performed using the PyroMark ID Pyrosequencing system (QIAGEN). First-round PCR was carried out followed by second-round PCR for T315I BCR-ABL mutation including one biotin-labeled primer. Primers and PCR conditions were used as described previously [20]. The linearity of quantitative T315I BCR-ABL mutation by pyrosequencing was confirmed by subjecting cDNA generated from graded mixes of Ba/F3 cell lines (RIKEN Cell Bank, Tsukuba, Japan) transfected with BCR-ABL cDNAs containing either the un-mutated BCR-ABL sequence or the T315I BCR-ABL mutation.

Case report

A 61-year-old male was referred to our hospital due to leukocytosis, thrombocytosis, and hepatosplenomegaly (hypochondrial spleen size 8 cm) in October 2002. Complete blood cell analysis showed that the white blood cell count was 138,900/μl, with 36% neutrophils, 3% myeloblasts, 5% promyelocytes, 5% myelocytes, 14% metamyelocytes, 6% lymphocytes, 5% monocytes, 5% basophils, and 3% eosinophils; hemoglobin concentration was 11.2 g/dl; and the platelet count was 122.1 × 10^4/μl. Bone marrow analysis showed hypercellularity with significant myeloid hyperplasia with 3.0% myeloblasts. Chromosomal analysis (G-banding) revealed that there were no additional chromosomal abnormalities other than t(9;22)(q34;q11). No BCR-ABL kinase domain mutation was detected by direct sequencing (Fig. 1a) and also by pyrosequencing. He was diagnosed with CP-CML. The Sokal score was 1.94, indicating high risk.

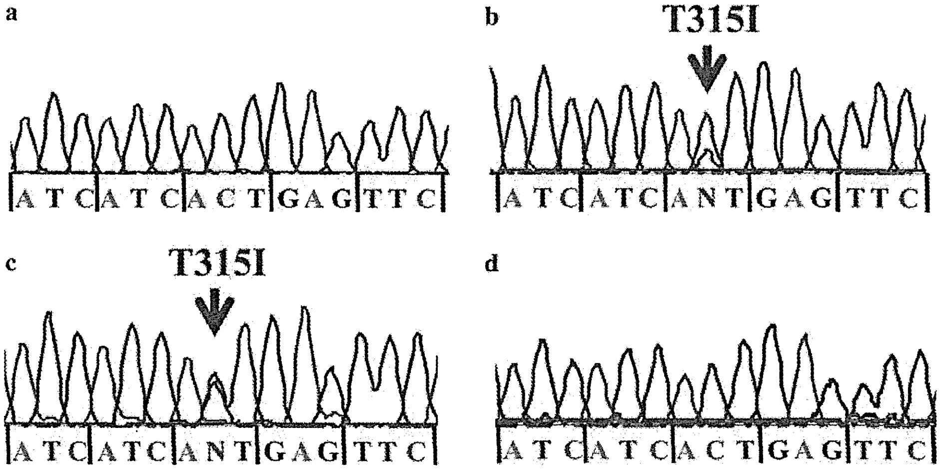

Fig. 1 T315I BCR-ABL mutation by direct sequencing: **a** at diagnosis, **b** at 18 months after starting imatinib, **c** at 24 months after starting imatinib, **d** at 51 months after starting the combination therapy

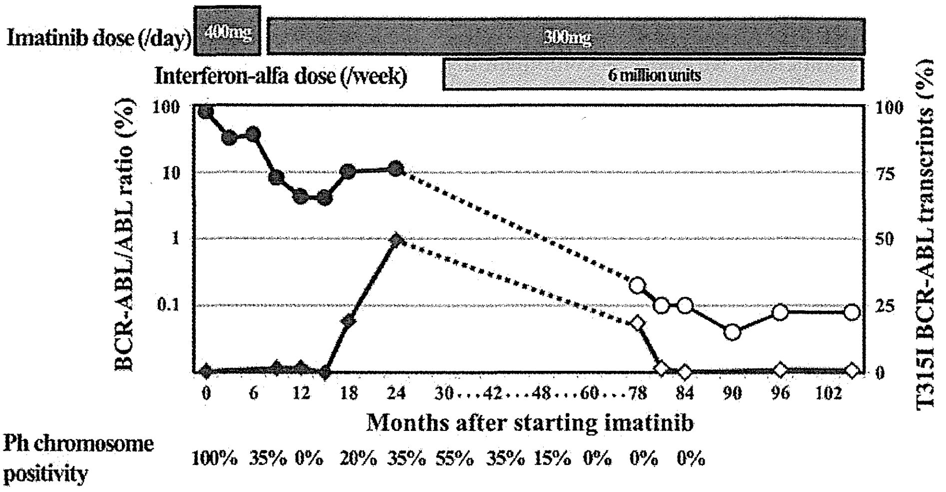

Fig. 2 Clinical course of total and T315I BCR-ABL mutant transcript levels. The figure shows total BCR-ABL transcript levels (*solid line*) measured by RQ-PCR and the relative size of T315I BCR-ABL mutant transcript levels (*dotted line*) by pyrosequencing. The *filled circle* and *filled square* represent samples from bone marrow, and the *open circle* and *open square* represent samples from peripheral blood. Ph chromosome positivity (%) represents the ratio of Ph-positive cells in bone marrow cells determined by G-band chromosomal analysis

He was registered in the clinical trial (Japan Adult Leukemia Study Group, CML202 study) and imatinib was initiated with a dose of 400 mg/day in October 2002. A dose reduction (300 mg/day) was necessary after 6 months due to muscle cramp, which was considered to be a side effect. Complete hematologic response (CHR) and complete cytogenetic response (CCyR) were achieved within 1 and 12 months of treatment, respectively. However, after 18 months of imatinib treatment, a loss of CCyR was observed and a direct sequencing study at 24 months revealed a T315I mutation of the BCR-ABL gene (Fig. 1b). The earlier samples (at 18 months) were then analyzed retrospectively and the mutation was also identified. Even though pyrosequencing revealed that T315I transcripts increased over 2.5-fold during the 18- to 24-month period (Fig. 1c), total BCR-ABL transcripts measured by a RQ-PCR remained unchanged: ratios of BCR-ABL to ABL were 10.1% at 18 months and 11.1% at 24 months, respectively. Because a loss of the major cytogenetic response occurred at 30 months, a combination therapy which consisted of imatinib and IFNα was initiated. IFNα was administrated at a dose of 6 million Units/week. Thirty months after the initiation of the imatinib/IFNα combination therapy, he re-achieved CCyR. Forty-eight months after, the T315I BCR-ABL mutation remained detectable although CCyR was maintained. After 51 months, RQ-PCR revealed a reduction of BCR-ABL transcripts by 3 or more logs [i.e., major molecular response (MMR)], and the T315I BCR-ABL mutation was not detected by direct sequencing and pyrosequencing (Fig. 1d). The MMR was still maintained at 75 months after the initiation of the imatinib/IFNα combination therapy without any signs of a recurrence of the T315I BCR-ABL mutation (Fig. 2). Although he experienced grade 2 anemia, grade 1 neutropenia, and thrombocytopenia according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0, it was possible to continue the imatinib/IFNα combination therapy with no dose reduction.

Discussion

The current treatment algorithm for patients with CML suggests that if the patient develops a T315I BCR-ABL mutation, allo-HSCT or participation in clinical trials should be considered (new agents against the T315I BCR-ABL mutation [15, 16, 21–24] are still in trials). In our case, the imatinib/IFNα combination therapy used resulted in MMR, suggesting its effectiveness in patients harboring the T315I BCR-ABL mutation. De Lavallade et al. [25] have reported the clinical outcome for a CML patient who acquired the T315 BCR-ABL mutation while on imatinib, that was treated successfully with IFNα alone. In their report, while the level of T315I BCR-ABL mutant transcripts decreased with the interferon therapy, the total amount of BCR-ABL transcripts was relatively stable, suggesting that the CML clone harboring an un-mutated BCR-ABL was expanding during that period. To prevent this phenomenon, we chose a combination therapy with imatinib and IFNα. This therapy theoretically seemed reasonable because it would inhibit both the T315I-mutated and the un-mutated BCR-ABL clone, and as shown in this report, it was quite successful. Determining whether or not the T315I BCR-ABL mutated clone is more susceptible to IFNα than an un-mutated clone would be of interest.

In conclusion, although our experience is limited to one patient, imatinib/IFNα combination therapy could be a viable treatment option for CP-CML patients with a T315I BCR-ABL mutation. Further studies are necessary to confirm the efficacy and applicability of imatinib/IFNα combination therapy.

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ORIGINAL ARTICLE

Efficacy and safety of nilotinib in Japanese patients with imatinib-resistant or -intolerant Ph+ CML or relapsed/refractory Ph+ ALL: a 36-month analysis of a phase I and II study

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Abstract Although the tyrosine kinase inhibitor (TKI) imatinib is often used as first-line therapy for newly diagnosed chronic myelogenous leukemia (CML), some patients fail to respond, or become intolerant to imatinib. Nilotinib is a potent and selective second-generation TKI, with confirmed efficacy and tolerability in patients with imatinib-resistant or -intolerant CML. A phase I/II study was conducted in Japanese patients with imatinib-resistant or -intolerant CML or relapsed/refractory Ph+ acute lymphoblastic leukemia. Thirty-four patients were treated with nilotinib for up to 36 months. Major cytogenetic response was achieved in 15/16 patients (93.8%) with chronic-phase CML within a median of approximately 3 months. Major molecular response was achieved in 13/16 patients (81.3%). These responses were sustained at the time of the most recent evaluation in 13 patients and 11 patients, respectively. Hematologic and cytogenetic responses were also observed in patients with advanced CML. The BCR-ABL mutation associated with the most resistance to available TKIs, T315I, was observed in three patients. Common adverse events included rash, nasopharyngitis, leukopenia, neutropenia, thrombocytopenia, nausea, headache and vomiting. Most adverse events resolved following nilotinib dose interruptions/reductions. These results support the favorable long-term efficacy and tolerability of nilotinib in Japanese patients with imatinib-resistant or -intolerant chronic-phase chronic myeloid leukemia.

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Introduction

The tyrosine kinase inhibitor (TKI) imatinib (ST1571, Glivec^{TM}; Novartis) has been shown to induce durable responses in a high proportion of patients with chronic-phase chronic myeloid leukemia (CML-CP) [1–5]. However, disease progression caused by resistance to imatinib occurs in some CML patients treated with this drug [6].

CML patients in the accelerated phase (CML-AP) or in blast crisis (CML-BC) also show a complete cytogenetic response (CCyR) following treatment with imatinib, but the proportion of such patients achieving CCyR is considerably lower than that of CML-CP patients [7, 8]. Moreover, imatinib resistance and relapse are also common in CML-AP and -BC patients [6, 9]. Imatinib is also used to treat patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL), and many of these patients also achieve CCyR. However, the CCyRs in these patients are not sustained for as long as they are in CML-CP patients, both in Japan [10] and in other countries [11].

Approximately half of the cases of imatinib resistance are now known to result from mutations in *BCR-ABL* [12–16], which make particular leukemic cells resistant to BCR-ABL tyrosine kinase inhibition by imatinib.

Nilotinib (AMN107, Tasigna®; Novartis) is a second-generation TKI that inhibits BCR-ABL-dependent cell proliferation and induces cell death in BCR-ABL phenotypic cells [17, 18]. Nilotinib was originally approved as second-line treatment for imatinib-resistant or -intolerant CML-CP and -AP patients [19–22]. More recently, it was approved as first-line therapy for CML-CP and -AP patients [23, 24] in Japan. Several studies have reported hematologic response (HR) and cytogenetic response (CyR) with nilotinib in patients with imatinib-resistant or -intolerant CML-BC and those with relapsed/refractory Ph+ ALL [25, 26].

We recently reported the results of a phase I and II study of nilotinib in which Japanese patients with imatinib-resistant or -intolerant Ph+ CML, or relapsed/refractory Ph+ ALL were treated for up to 12 months [22]. Here, we report the effects of treatment with nilotinib for up to 36 months in these patients, as well as the results of mutation analysis and the response by BCR-ABL mutation status.

Materials and methods

Study design and objectives

This was an open-label, multicenter, continuous-dose, 36-month extension of a phase I and II clinical study. The study protocol and documentation were approved by the institutional review boards of each participating center. The observation period was defined to be 36 months, including the entire 3 months of the Ph I/II clinical study. The study was conducted in accordance with the ethical principles established by the Declaration of Helsinki and in compliance with institutional guidelines.

The primary objective of this extension study was to evaluate the long-term safety of nilotinib, including chronic toxicity. Secondary objectives included the long-term efficacy of nilotinib, the relationship between BCR-ABL mutations or BCR-ABL transcript levels determined by quantitative RT-PCR, and the clinical efficacy of nilotinib. The time of last evaluation in this study was the time at which patients had received treatment for more than 3 years or the time at which the drug became commercially available at each of the study institutions, whichever was the later.

Patients

The inclusion and exclusion criteria are described in the original study report [22]. Briefly, Japanese patients were eligible if they had imatinib-resistant or -intolerant CML-CP, CML-AP, CML-BC or relapsed/refractory Ph+ ALL, were at least 20 years of age, had a World Health Organization (WHO) performance status (PS) ≤ 2, and had normal hepatic, renal and cardiac function.

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Treatments

Nilotinib 400 mg was administered orally twice daily. Patients were required to fast for 2 h before and after each dose. One treatment course (1 cycle) was defined as 28 consecutive days of twice-daily nilotinib. If administration was delayed for more than 21 days (42 days for hematologic toxicity) after the previous dose, the patient was withdrawn from the study. Dose reductions to 400 mg once daily (one level lower than the standard dose) or 200 mg once daily (two levels lower than the standard dose) were permitted. The nilotinib dose at re-introduction was one level lower than that at cessation. The mean dose in each patient was calculated by assuming the dose during the cessation period to be 0 mg.

Treatment with nilotinib was continued until disease progression or unacceptable toxicity was observed, or at the investigator's discretion that treatment be discontinued. After the regulatory approval date for nilotinib in Japan (January 29, 2009), its administration was continued for longer than 3 years or until the drug became commercially available, whichever was later.

Measurements

Response rates

Criteria for HR and CyR were similar to those reported elsewhere [19, 21, 27] and are described in more detail in Tojo et al. [22]. Briefly, CyR was determined as the percentage of Ph+ cells of $\geq$20 cells in the metaphase in each bone marrow sample, and was classified as complete (0% Ph+ cells), partial (1–35% Ph+ cells), minor (36–65% Ph + cells) or minimal (66–95% Ph+ cells). Major CyR (MCyR) included complete and partial CyR. Fluorescent in situ hybridization was used if <20 cells were examined or if the bone marrow sample was not adequate for assessment.

The proportion of patients who experienced major molecular response (MMR) was also determined for each disease phase and subtype. BCR-ABL transcript levels were measured by quantitative RT-PCR and reported in the international scale using a conversion factor of 1.25 established by the Institute of Medical and Veterinary Science, Australia. MMR was defined as a BCR-ABL/BCR ratio $\leq$0.1%. Loss of MMR was defined as a BCR-ABL/BCR ratio >0.1%. Patients with MMR at baseline were considered "not evaluable" and were excluded from the analysis. Only evaluable patients in the intention-to-treat (ITT) population were included in the analyses of overall response rates.

Patients whose BCR-ABL transcript levels were not evaluated at baseline were considered "not assessable", and were not included in the denominator when calculating the proportion of patients who achieved MMR.

Mutation analysis

Efficacy was also examined based on the subtype of BCR-ABL mutation at baseline and after nilotinib administration. Mutation analysis was performed by the direct sequence identification method. The number and proportion of patients with HR, CyR or MMR were calculated for the following categories of mutation [22]: no mutation, any mutation, multiple mutations, P-loop mutations (amino acids 248–255), non-P-loop mutations, and protocol-specified subgroup mutations associated with imatinib resistance mutations (L248, Q252, T315, F317, H396, M237, M244, G250, D325, S348, M351, E355, A380, L387, M388, F486, Y253, E255, and F359). The impact of baseline mutations or development of new mutations on patient outcomes was assessed.

Safety analyses

Safety assessment included an evaluation of the frequency and severity of adverse events, which included hematologic and biochemical laboratory tests, vital signs, physical examinations (including body weight), WHO PS, cardiac function tests (12-lead ECG, cardiac enzyme test, echocardiography), and chest X-rays, as needed. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). The monitoring was continued for at least 28 days after the last dose of nilotinib.

Statistical analyses

The ITT population was used for the efficacy analysis and was pre-specified as all patients enrolled in either the phase I or phase II studies, and who were treated with nilotinib 400 mg twice daily, irrespective of when they withdrew from the study. The safety (SAF) population comprised all patients in the ITT population who underwent safety assessments. HR, CyR and MMR were summarized by disease phase and subtype (CML-CP, CML-AP, CML-BC, and Ph+ ALL). The time to first response and duration of response were assessed by descriptive statistics or Kaplan–Meier analysis, as appropriate. No statistical comparisons were made.

Results

Patients and treatment administration

This 36-month study included 34 Japanese patients with imatinib-resistant or -intolerant CML (CML-CP, $N = 16$; CML-AP, $N = 7$; CML-BC, $N = 4$) or Ph+ ALL ($N = 7$).

Thirty-one patients were enrolled into the phase II study and treated with nilotinib 400 mg twice daily (CML-CP: 14, CML-AP: 7, CML-BC: 3; Ph+ ALL: 7) and 3 patients were enrolled in the phase I study and treated with nilotinib 400 mg twice daily (CML-CP: 2; CML-BC: 1) [22]. The characteristics and disposition of patients are summarized in Tables 1 and 2, respectively. Fourteen patients (CML-CP: 13; CML-AP: 1) received nilotinib until the end of the study while 20 patients (CML-CP: 3, CML-AP: 6, CML-BC: 4; Ph+ ALL: 7) discontinued study treatment. The

Table 1 Patient characteristics (ITT population)

	CML-CP ($N = 16$)	CML-AP ($N = 7$)	CML-BC ($N = 4$)	Ph+ ALL ($N = 7$)	Total ($N = 34$)
Age (years)	57.0 (30–83)	61.0 (30–74)	53.0 (29–70)	62.0 (23–80)	61.5 (23–83)
Sex					
Male	9 (56)	5 (71)	2 (50)	6 (86)	22 (65)
Female	7 (44)	2 (29)	2 (50)	1 (14)	12 (35)
Body weight (kg)	61.2 (44.5–89.0)	64.8 (49.1–83.0)	63.3 (35.5–69.0)	55.8 (46.2–60.2)	60.5 (35.5–89.0)
WHO PS					
0	16 (100)	4 (57)	2 (50)	4 (57)	26 (76)
1	0 (0)	2 (29)	2 (50)	3 (43)	7 (21)
2	0 (0)	1 (14)	0 (0)	0 (0)	1 (3)
Time since first diagnosis (months)	30.4 (1.4–122.8)	108.6 (12.5–192.8)	65.3 (20.5–102.8)	16.2 (3.7–134.1)	30.4 (1.4–192.8)
Imatinib resistance	4 (25.0)	4 (57.1)	4 (100.0)	7 (100.0)	19 (55.9)
Imatinib intolerance	12 (75.0)	3 (42.9)	0 (0.0)	0 (0.0)	15 (44.1)
Highest imatinib dose (mg)	500 (200–800)	800 (400–800)	700 (600–800)	600 (600–600)	600 (200–800)

Values are *n* (%) or median (range)

ITT intention-to-treat, *WHO PS* World Health Organization performance status

Table 2 Patient disposition (ITT population)

	CML-CP ($N = 16$)	CML-AP ($N = 7$)	CML-BC ($N = 4$)	Ph+ ALL ($N = 7$)	Total ($N = 34$)
Completed the long-term study	13 (81)	1 (14)	0 (0)	0 (0)	14 (41)
Discontinued treatment and withdrawn from the study	3 (19)	6 (86)	4 (100)	7 (100)	20 (59)
Reason for discontinuation					
Adverse event(s)	0 (0)	1 (14)	1 (25)	1 (14)	3 (9)
Allo-HSCT performed	1 (6)	2 (29)	1 (25)	0 (0)	4 (12)
Disease progression	1 (6)	3 (43)	2 (50)	6 (86)	12 (35)
Withdrawal of consent	1 (6)	0 (0)	0 (0)	0 (0)	1 (3)
Dose reduction	15 (94)	5 (71)	3 (75)	4 (57)	27 (79)
Withdrawal from treatment	11 (69)	2 (29)	2 (50)	2 (29)	17 (50)
Drug administration recommenced at a reduced dose after withdrawal	10 (63)	1 (14)	0 (0)	2 (29)	13 (38)
Duration of exposure (days)[a]	1099.5 (176–1173)	84.0 (56–1099)	133.0 (15–247)	56.0 (13–644)	445.5 (13–1173)
Duration of administration (days)[b]	1084.5 (165–1173)	84.0 (28–1099)	126.5 (14–247)	56.0 (13–609)	428.0 (13–1173)
Daily dose (mg)[c]	612.9 (394.2–798.6)	789.6 (284.9–797.5)	742.6 (402.4–798.4)	785.7 (483.2–794.1)	750.7 (284.9–798.6)

Values are *n* (%) or median (range)

Allo-HSCT allogeneic hematopoietic stem cell transplantation, *ITT* intention-to-treat

[a] Includes drug interruptions

[b] Excludes drug interruptions

[c] Daily dose = total dose/duration of exposure (includes drug interruption)

Table 3 Best responses to nilotinib (ITT population)

	CML-CP ($N = 16$)	CML-AP ($N = 7$)	CML-BC ($N = 4$)	Ph+ ALL ($N = 7$)
Hematologic response (HR)	6 (100)[a]	5 (71)	2 (50)	3 (43)
Complete hematologic response	6 (100)	1 (14)	1 (25)	–
Complete response	–	–	–	3 (43)
Marrow response with no evidence of leukemia	–	3 (43)	0 (0)	–
Return to chronic phase	–	1 (14)	1 (25)	–
Stable disease	0 (0)	1 (14)	2 (50)	1 (14)
Progressive disease	0 (0)	0 (0)	0 (0)	3 (43)
Not evaluable/not assessable	10 (63)	1 (14)	0 (0)	0 (0)
Cytogenetic response (CyR)				
Major	15 (94)	1 (14)	2 (50)	–
Complete	13 (81)	1 (14)	2 (50)	–
Partial	2 (13)	0 (0)	0 (0)	–
Minor	0 (0)	0 (0)	1 (25)	–
Minimal	1 (6)	3 (43)	0 (0)	–
None	0 (0)	1 (14)	0 (0)	–
Not assessable	0 (0)	2 (29)	1 (25)	–
Molecular response (MR)				
Major[b]	13 (81)	1 (14)	2 (50)	1 (17)[c]
None	3 (19)	6 (86)	2 (50)	5 (83)[c]
Not evaluable	0 (0)	0 (0)	0 (0)	1 (14)

Values are *n* (%)

[a] Of which 6 were evaluable

[b] Major molecular response was defined as a BCR-ABL/BCR ratio ≤0.1%

[c] Of which 6 were evaluable

ITT intention-to-treat

most frequent reason for discontinuation was disease progression in 12 patients. Disease progression was seen in 1 patient with CML-CP, 3 patients with CML-AP, 2 patients with CML-BC and 6 patients with Ph+ ALL.

The median duration (range) of nilotinib exposure was 445.5 days (13–1173 days) and that of administration was 428.0 days (13–1173 days). The median daily dose (range) of nilotinib was 750.7 mg/day (284.9–798.6 mg/day) in all patients, consistent with the planned dose of administration (400 mg twice daily = 800 mg/day) in the study protocol. Dose reductions occurred in 27 patients (79.4%) because of adverse events in 19 patients (55.9%), in accordance with the study protocol in 14 patients (41.2%), incorrect administration in 10 patients (29.4%) or incorrect scheduling in 1 patient (2.9%) (multiple dose reductions were possible). Treatment interruption occurred in 17 patients (50.0%) because of adverse events in all 17 patients. Thirteen of these patients showed improvement of adverse events and were able to restart nilotinib administration at a lower dose.

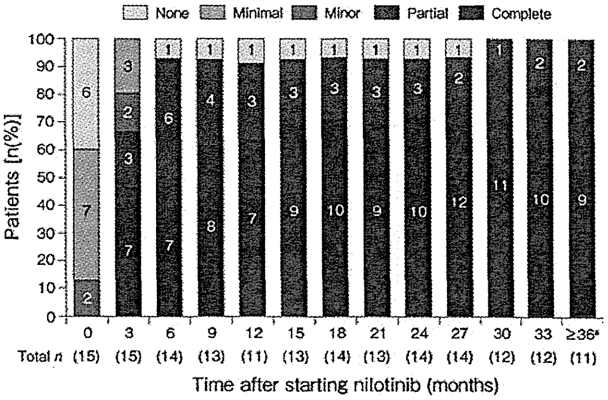

Fig. 1 Cytogenetic responses in CML-CP patients. [a]Including up to and beyond 36 months

Efficacy

CML-CP

The best responses (HR, CyR and MR) in the ITT population are shown in Table 3. All 6 CML-CP patients without CHR at baseline achieved CHR. The median time (range) to CHR was 28 days (28–56 days). Of these, 5 patients showed sustained response up to the last evaluation, while the remaining patient discontinued treatment on Day 787 because of disease progression. The duration of CHR in that patient was 478 days. MCyR was achieved in 15 patients (93.8%) and the response was sustained at the last evaluation in 13 patients. CCyR was achieved in 13 patients (81.3%) and the response was sustained at the last evaluation in 11 patients. The median time (range) to MCyR or CCyR was 84 days (28–178 days) and 97 days (57–847 days), respectively. The rate of CyR in evaluable patients at each time point is shown in Fig. 1. Thirteen

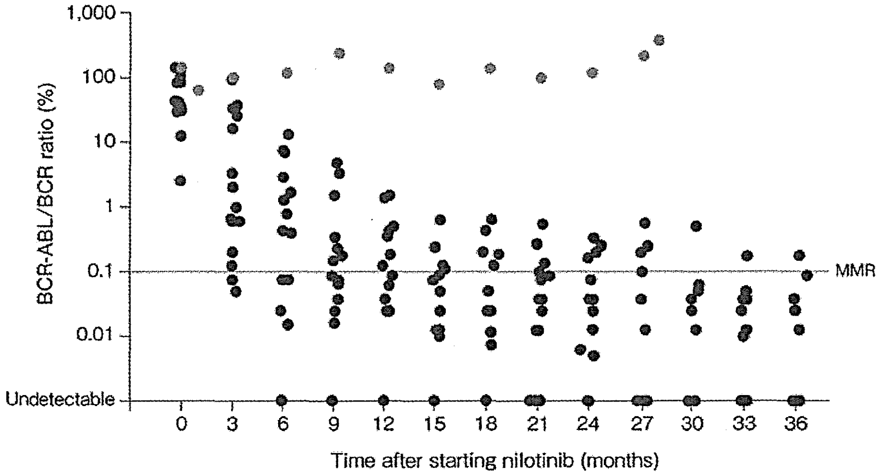

Fig. 2 Molecular responses during the 36-month study in patients with CML-CP. *MMR* major molecular response

patients continued treatment at 36 months or later. Among them, 11 patients were evaluated as showing cytogenetic response, all of whom achieved MCyR, including 9 with CCyR. The figure shows that the proportion of CCyRs increased with nilotinib treatment period.

The BCR-ABL/BCR ratio in CML-CP patients over time is shown in Fig. 2. The BCR-ABL/BCR ratio gradually decreased from baseline with long-term nilotinib treatment in all patients except one with baseline or newly detected mutations. An approximately 1-log reduction in BCR-ABL/BCR ratio from baseline at 6 months and an approximately 2-log reduction at 12 months were observed. MMR was achieved in 13 patients (81.3%) and was sustained at the last evaluation in 11 patients. The median time (range) to MMR was 248 days (84–852 days) in these CML-CP patients.

Among CML-CP patients, 3 patients discontinued nilotinib treatment. One patient discontinued treatment on Day 176 to undergo allogeneic hematopoietic stem cell transplantation (allo-HSCT). Another patient once achieved CCyR but discontinued treatment on Day 787 because of disease progression, as mentioned above. This patient had a newly detected mutation (F359V). Another patient withdrew consent on Day 931.

CML-AP

Among 7 CML-AP patients, 5 patients (71.4%) achieved HR, including CHR in 1 patient, marrow response with no evidence of leukemia in 3 patients, and return to chronic phase in 1 patient. Of the remaining 2 patients, 1 had stable disease and 1 was not evaluable. Of the 5 patients with HR, 1 patient with CHR and another 2 patients with HR experienced sustained response at the last evaluation or at discontinuation of treatment. In the remaining 2 patients, the duration of HR was 29 and 57 days, respectively. Minimal CyR was observed in 3 patients (42.9%). One patient with CHR achieved CCyR (14.3%). This patient also achieved MMR, which was sustained at the last evaluation.

CML-BC

Among 4 CML-BC patients, 2 patients (50.0%) achieved HR, including CHR in 1 patient and return to chronic phase in 1 patient. They also achieved CCyR and MMR. In both patients, MCyR was sustained until discontinuation of treatment to undergo allo-HSCT (on Day 247) in the first patient, or because of increasing blast numbers in bone marrow (on Day 168) in the second patient. The remaining 2 patients (50.0%) experienced stable disease and one of them achieved minor CyR.

Ph+ ALL

Among 7 patients with relapsed/refractory Ph + ALL, 1 of 5 patients (20.0%) without MRD experienced HR (complete response [CR]), which was sustained for 108 days. Three patients experienced disease progression and 1 experienced stable disease. Both patients with MRD achieved HR (CR). In one of these patients, CR was sustained for 58 days, but treatment was discontinued on Day 109 because of encephalitis. In the other patient, CR was sustained for 470 days, but treatment was discontinued on Day 644 because of disease progression. MMR was achieved in 1 patient with MRD, while the other patient with MRD achieved MMR at baseline and was thus considered not evaluable.

BCR-ABL mutations

Detection of new mutations

The development of new BCR-ABL mutations during the administration of nilotinib in this study is shown in

Table 4 Detection of new BCR-ABL mutations

Stage	Mutation	Day of detection	Baseline mutation	Achieved MMR	Outcome
CML-CP	F359V	174	M244V	No	Disease progression
CML-CP	E255K	340	None	Yes	Continued
CML-BC	T315I/Y253H	168	F317L	Yes	Disease progression
Ph+ ALL	T315I	16	E255K/E255V/G250E	No	Disease progression
Ph+ ALL	E255V	57	E459K	No	Disease progression
Ph+ ALL	T315I	43	None	No	Disease progression
Ph+ ALL	E255K/E255V	135	NA	No	Disease progression

MMR major molecular response, *NA* Not assessable

Table 4. New mutations were detected in 7 patients during nilotinib treatment. Among them, the T315I mutation occurred in 3 patients and nilotinib was discontinued in these patients because of disease progression. Three of the 4 patients with mutations other than T315I also discontinued treatment because of disease progression. The remaining patient continued treatment.

CML-CP

Among 16 CML-CP patients, MMR was observed in 4 of 5 patients (80.0%) with BCR-ABL mutations at baseline or emerging during the treatment period. As shown in Table 4, new mutations were detected in 2 patients.

One patient had a baseline M244V mutation and achieved minimal CyR on Day 87; however, an F359V mutation was also detected on Day 174. From Day 426, only the F359V mutation was detected and the M244V mutation was not; this patient was withdrawn from the study because of disease progression on Day 787 (see "CML-CP" under the heading Efficacy). In another patient without baseline mutation, E255K was detected only once on Day 340. This patient achieved MMR on Day 511, which was sustained at the last evaluation, and the mutation was not detected again after achievement of MMR. In 1 patient with an imatinib resistance-associated mutation (F359I) at baseline, the mutation could not be detected after commencing nilotinib treatment, which led to MMR that had been sustained for 666 days at the last evaluation.

CML-AP/-BC and Ph+ ALL

Among 7 CML-AP patients, no new mutations were detected. As shown in Table 4, among 4 CML-BC patients, new mutations were detected in 1 patient with the F317L mutation at baseline. This patient achieved CCyR and MMR on Day 56; however, Y253H and T315I mutations were detected on Day 168 followed by disease progression on Day 171. Among 7 Ph+ ALL patients, new mutations were detected in 4 patients, all of whom experienced disease progression.

Safety analysis

All adverse events regardless of drug relationship occurring at a frequency ≥20% and those of grade 3/4 are summarized in Table 5 (adverse events and adverse drug reactions occurring in ≥10% of subjects are shown in Supplemental Tables 1 and 2, respectively, while all adverse events of grade 3 or worse are shown in Supplemental Table 3). Adverse events occurred in all of the patients. The most common non-hematologic events were rash (64.7%), nasopharyngitis (58.8%), nausea and headache (47.1% each), and vomiting (41.2%). Hematologic events included leukopenia (47.1%), neutropenia (47.1%), thrombocytopenia (47.1%) and anemia (38.2%).

Adverse events of grade 3/4 occurred in 29/34 patients (85.3%). The most frequent grade 3/4 non-hematologic events were abnormal hepatic function, hyponatremia and pneumonia (11.8% each). Grade 3/4 hematologic events included neutropenia (47.1%), leukopenia (41.2%), thrombocytopenia (32.4%), anemia (29.4%) and lymphopenia (11.8%). The most common biochemical grade 3/4 events were decreased blood phosphorus levels (14.7%), hyperglycemia and increased lipase levels (11.8% each).

Serious adverse events

Thirty-four serious adverse events occurred in 19 patients. Among these, 21 events in 12 patients were considered possibly related to nilotinib. Two of these patients discontinued nilotinib treatment because of serious adverse events considered to be related to the drug. One, with CML-BC, developed back pain (non-serious) and discontinued treatment. Two days later, this patient developed cardiac tamponade and pericardial effusion, and died because of heart failure. The other, with Ph+ ALL, developed encephalitis and also discontinued treatment. Furthermore, one CML-CP patient developed acute pancreatitis reported as a serious adverse event that resolved

Table 5 Non-hematologic, hematologic and biochemical adverse events with a frequency ≥20% for all grades

Total $N = 34$	All grades					Grade 3/4				
	CML-CP *n* (%)	CML-AP *n* (%)	CML-BC *n* (%)	Ph+ ALL *n* (%)	Total *n* (%)	CML-CP *n* (%)	CML-AP *n* (%)	CML-BC *n* (%)	Ph+ ALL *n* (%)	Total *n* (%)
Non-hematologic events										
Rash	9 (56.3)	5 (71.4)	3 (75.0)	5 (71.4)	22 (64.7)	1 (6.3)	0 (0.0)	1 (25.0)	0 (0.0)	2 (5.9)
Nasopharyngitis	15 (93.8)	3 (42.9)	2 (50.0)	0 (0.0)	20 (58.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	7 (43.8)	2 (28.6)	3 (75.0)	4 (57.1)	16 (47.1)	0 (0.0)	0 (0.0)	1 (25.0)	1 (14.3)	2 (5.9)
Nausea	6 (37.5)	3 (42.9)	4 (100.0)	3 (42.9)	16 (47.1)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (2.9)
Vomiting	6 (37.5)	3 (42.9)	2 (50.0)	3 (42.9)	14 (41.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pyrexia	4 (25.0)	1 (14.3)	4 (100.0)	4 (57.1)	13 (38.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Constipation	8 (50.0)	2 (28.6)	1 (25.0)	1 (14.3)	12 (35.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperbilirubinemia	5 (31.3)	3 (42.9)	1 (25.0)	1 (14.3)	10 (29.4)	2 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.9)
Hyperglycemia	8 (50.0)	1 (14.3)	1 (25.0)	0 (0.0)	10 (29.4)	2 (12.5)	1 (14.3)	1 (25.0)	0 (0.0)	4 (11.8)
Malaise	8 (50.0)	0 (0.0)	0 (0.0)	2 (28.6)	10 (29.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Back pain	6 (37.5)	0 (0.0)	2 (50.0)	1 (14.3)	9 (26.5)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (2.9)
Pruritus	3 (18.8)	2 (28.6)	1 (25.0)	3 (42.9)	9 (26.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abnormal hepatic function	5 (31.3)	0 (0.0)	1 (25.0)	2 (28.6)	8 (23.5)	1 (6.3)	0 (0.0)	1 (25.0)	2 (28.6)	4 (11.8)
Conjunctivitis	7 (43.8)	1 (14.3)	0 (0.0)	0 (0.0)	8 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhea	3 (18.8)	2 (28.6)	1 (25.0)	2 (28.6)	8 (23.5)	0 (0.0)	0 (0.0)	1 (25.0)	1 (14.3)	2 (5.9)
Anorexia	5 (31.3)	1 (14.3)	0 (0.0)	1 (14.3)	7 (20.6)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Arthralgia	5 (31.3)	2 (28.6)	0 (0.0)	0 (0.0)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Eczema	6 (37.5)	0 (0.0)	1 (25.0)	0 (0.0)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypokalemia	1 (6.3)	2 (28.6)	2 (50.0)	2 (28.6)	7 (20.6)	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	2 (5.9)
Insomnia	2 (12.5)	2 (28.6)	1 (25.0)	2 (28.6)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pharyngitis	4 (25.0)	0 (0.0)	0 (0.0)	3 (42.9)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hematologic events										
Leukopenia	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)	5 (31.3)	3 (42.9)	2 (50.0)	4 (57.1)	14 (41.2)
Neutropenia	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)
Thrombocytopenia	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)	3 (18.8)	3 (42.9)	2 (50.0)	3 (42.9)	11 (32.4)
Anemia	5 (31.3)	2 (28.6)	3 (75.0)	3 (42.9)	13 (38.2)	3 (18.8)	2 (28.6)	2 (50.0)	3 (42.9)	10 (29.4)
Biochemical events										
Increased bilirubin	6 (37.5)	1 (14.3)	1 (25.0)	2 (28.6)	10 (29.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Increased alanine aminotransferase	3 (18.8)	0 (0.0)	2 (50.0)	3 (42.9)	8 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	1 (2.9)
Increased lipase	5 (31.3)	1 (14.3)	1 (25.0)	1 (14.3)	8 (23.5)	3 (18.8)	1 (14.3)	0 (0.0)	0 (0.0)	4 (11.8)

The table includes drug-related and non-related adverse events combined

following nilotinib dose interruption. This patient restarted nilotinib at 400 mg once daily, which was then increased to 400 mg twice daily, and the subject completed study treatment. QT interval prolongation occurred in 1 CML-CP patient and nilotinib treatment was interrupted. This patient restarted nilotinib at 400 mg once daily and continued treatment without QT interval prolongation.

Adverse events by time-points

Among the CML-CP patients, the incidences of blood/lymphatic system disorders, gastrointestinal disorders, laboratory abnormalities, and skin/subcutaneous tissue disorders in Cycles 1–12 in the first year of treatment were 68.8, 87.5, 62.5 and 75.0%, respectively. The incidences of these events were much lower during Cycles 13–24 (20.0, 40.0, 40.0 and 53.3%, respectively) and Cycles 25 or later (20.0, 73.3, 26.7, 46.7%) in the second year of treatment. Gastrointestinal disorders showed higher incidence in Cycles 25 or later (3 years or more of treatment) and, in particular, the incidence of constipation was as high as 26.7%. Fewer patients with CML-AP, CML-BC, and Ph+ ALL continued treatment beyond Cycle 24, so no significant difference in the