

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

Outcome	Univariate analysis		Multivariate analysis	
	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).

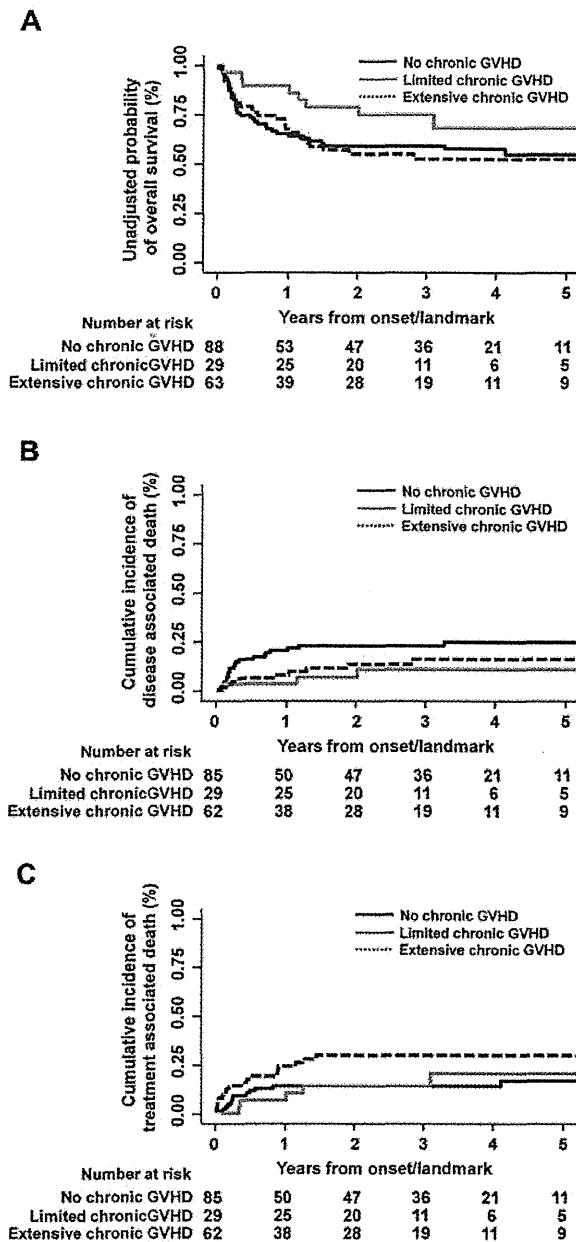


Figure 3. Semi-landmark plots for impact of chronic GVHD. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

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

Outcome	Univariate analysis		Multivariate analysis	
	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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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.

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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 (IFN α) 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/IFN α combination therapy for CML patients bearing the T315I BCR-ABL mutation.

Keywords Chronic myelogenous leukemia · Imatinib · Interferon · T315I

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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 IFN α [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

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 IFN α .

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'-ACAGGATCAACACTGCTTCTGA-3'); ABL-1R (5'-TGGCTGACGAGATCTGAGTG-3'); ABL-2F (5'-ATGGCCACTCAGATCTCGTC-3'); and ABL-2R (5'-GATACTGGATTCTGGAACA-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/ μ l, 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 / μ l. 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

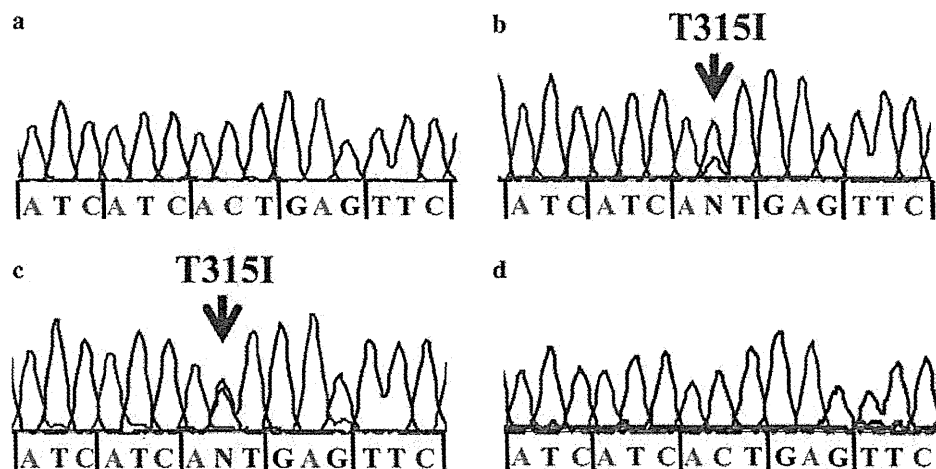
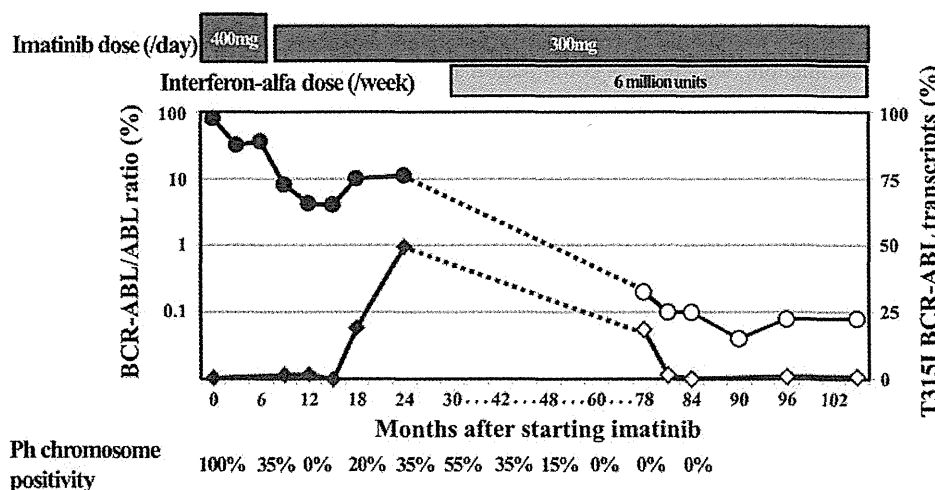


Fig. 2 Clinical course of total and T315I BCR-ABL mutant transcript levels. The figure shows total BCR-ABL transcript levels (solid line) measured by RQ-PCR and the relative size of T315I BCR-ABL mutant transcript levels (dotted line) by pyrosequencing. The filled circle and filled square represent samples 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 IFN α was initiated. IFN α was administered at a dose of 6 million Units/week. Thirty months after the initiation of the imatinib/IFN α 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/IFN α 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/IFN α 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/IFN α 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 IFN α 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 IFN α . 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 IFN α than an un-mutated clone would be of interest.

In conclusion, although our experience is limited to one patient, imatinib/IFN α 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/IFN α combination therapy.

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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, GlivecTM; 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 III 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 ≥ 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.

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

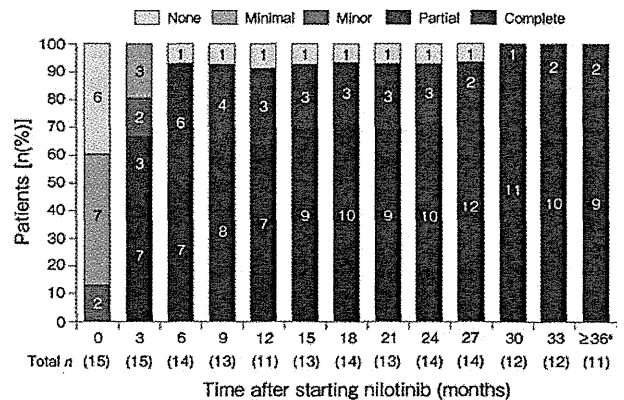
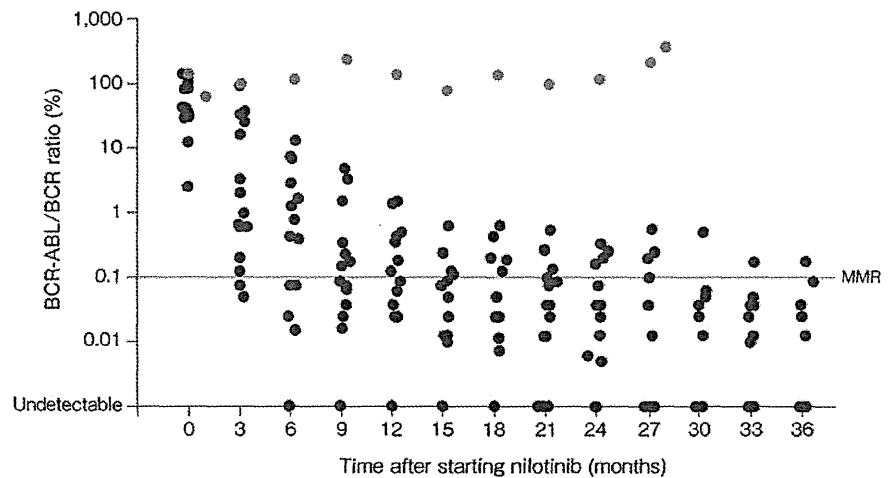


Fig. 1 Cytogetic responses in CML-CP patients. ^aIncluding up to and beyond 36 months

(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 $\geq 20\%$ and those of grade 3/4 are summarized in Table 5 (adverse events and adverse drug reactions occurring in $\geq 10\%$ 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 $\geq 20\%$ 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

incidence of these adverse events between time-points was observed.

Discussion

Here, we report the long-term efficacy and tolerability profiles of nilotinib in 34 patients with imatinib-resistant or -intolerant Ph+ CML or relapsed/refractory Ph+ ALL. In comparison with the findings obtained at 12 months [22], there were few occurrences of new adverse events during the 36-month study.

In the phase I/II clinical trial of nilotinib [22], the drug was found to be generally safe and well-tolerated in patients with imatinib-resistant or -intolerant CML, and those with relapsed/refractory Ph+ ALL. The tolerability of nilotinib up to doses of 400 mg twice daily was confirmed in Japanese patients. The dose intensity of nilotinib increased with increasing dose within the investigated dose range, and the 400 mg twice-daily dose regimen gave the highest exposure.

In the present extension study, in CML-CP patients, CCyR was achieved in 13/16 patients (81.3%) and CCyR was achieved rapidly, within a median of approximately 3 months. Furthermore, MMR (defined as a BCR-ABL/BCR ratio $\leq 0.1\%$) was also achieved in 13/16 patients (81.3%). These results compare favorably with those reported after 24 months of nilotinib treatment in another study of imatinib-resistant or -intolerant CML-CP [20]. In that study, 44% (141/321) of patients achieved CCyR and 28% (82/294) of patients achieved MMR. Comparable rates of HR, CyR and MMR during nilotinib therapy in CML-CP were reported in other studies. In this analysis, 13/16 patients achieved MMR and, in some patients, the BCR-ABL transcript level was undetectable by quantitative RT-PCR.

One CML-AP patient who responded well to nilotinib and achieved CCyR was treated with nilotinib for 3 years. This suggests that nilotinib has long-term benefits for the treatment of some patients with CML-AP. The findings in Ph+ ALL and CML-BC patients in this study are similar to those reported in other studies [26]. Although the sample size is small, the results obtained in 4 CML-BC patients and 7 Ph+ ALL patients suggest that, in some patients, nilotinib may be an effective drug for the treatment of imatinib-resistant or -intolerant CML-BC and Ph+ ALL. Further studies are needed in patients with advanced CML to verify these results. All 5 Ph+ ALL patients without MRD in this study were previously treated with imatinib, and only 1 patient (20.0%) achieved HR. The other 4 patients ultimately discontinued treatment because of disease progression. In contrast, both Ph+ ALL patients with MRD achieved HR. The small sample size in this study

meant that patients with imatinib-resistant or -intolerant disease were considered together, not separately.

As reported previously [28], imatinib resistance or intolerance, or the presence of baseline BCR-ABL mutations associated with imatinib resistance, did not affect the response to nilotinib. We detected 5 new mutations in 7 patients after starting nilotinib treatment. T315I, which is the mutation associated with the most resistance to currently available TKIs, was detected in 3 patients (8.8%) with CML-BC or Ph+ ALL; these patients discontinued treatment because of disease progression. Three of the 4 patients who developed other mutations also discontinued treatment, and the remaining patient, who had an E255K mutation, achieved MMR. These findings are consistent with previous studies suggesting that patients with the T315I mutation have a poor response to nilotinib [12, 19].

Two types of amino acid substitution at F359, F359V and F359I, were detected in this study. A CML-CP patient with baseline M244V mutation later harbored an F359V mutation (detected on Day 174) and showed poor response to nilotinib treatment; this patient experienced disease progression, as seen in other patients with the F359V mutation described in previous reports [29]. On the other hand, another patient who had F359I mutation at baseline achieved MMR. A previous study [30] showed that the F359I mutation is moderately sensitive to nilotinib (IC_{90} value = 433 nM). Nevertheless, in the present study, nilotinib treatment was effective, and sustainable MMR was observed in the patient with F359I mutation at baseline.

A recent study also described that CML patients with baseline mutations on imatinib treatment were more likely to relapse because of the development of other mutations after receiving dasatinib or nilotinib as second-line treatment [31]. Although the sample size of our study was small, only one CML-CP patient with a BCR-ABL mutation showed disease progression while the others completed study treatment. The effects of BCR-ABL mutation on the efficacy of treatment may differ depending on not only the type of mutation, but also the disease type and stage.

Adverse events of any grade occurred in all of the patients, regardless of drug relationship, and adverse events of grade 3/4 occurred in 29/34 patients (85.3%). The most common hematologic or non-hematologic adverse events included rash, nasopharyngitis, nausea, headache, vomiting, leukopenia, neutropenia and thrombocytopenia. Hematologic adverse events were commonly of grade 3/4 severity, similar to previously reported findings [19–21, 25, 26, 28]. Abnormal biochemical findings included hyperbilirubinemia, hyperglycemia and increased lipase. The rates of abnormal hematologic/blood biochemical findings were similar to those reported in a 12-month study [22] and in a global phase II study [19–21]. Most of these events

were not serious. The majority of adverse events did not require treatment discontinuation, interruption or dose reduction. Taken together, these findings are comparable with those reported in global phase I and II clinical studies [19–21, 25, 26] and a retrospective multicenter analysis [28]. During the 36-month observation period, only one patient with CML-BC died. Death resulted from heart failure due to cardiac tamponade and pericardial effusion occurring after discontinuation of nilotinib treatment.

Hematological and cytogenetic effects of nilotinib have been already observed in studies of up to 12 months [22] or 24 months in duration [20]. We have extended these findings in Japanese patients with imatinib-resistant or -intolerant Ph+ CML (CP, AP, or BC) or relapsed/refractory Ph+ ALL treated with nilotinib 400 mg twice daily for up to 36 months in this study. Importantly, nilotinib was shown to be effective as a second-line treatment for patients who failed to respond to previous imatinib treatment and who were considered to have a poor prognosis, with many patients achieving HR and CyR, which were maintained until last observation. No safety concerns arose over 36 months of treatment that were not apparent during the first 12 months of treatment. Most adverse events resolved following nilotinib dose interruption, dose reduction or supportive care.

The median daily dose of nilotinib (750.7 mg; range 284.9–798.6 mg) was below the prescribed dose (800 mg), mainly as a result of dose reductions in response to adverse events. In a previous study of nilotinib in Japanese newly diagnosed CML patients [24], the median dose was 730 mg (range, 644–794 mg) in the group administered nilotinib 400 mg twice daily; this dose was not considered particularly low, providing dose intensities similar to those in the overall population. The dose reduction in that study [24] was similar to that in ours.

Nilotinib was approved in Japan for the treatment of patients with CML-CP or CML-AP, but not patients with CML-BC or Ph+ ALL. The results of this study update provide further evidence supporting the use of nilotinib in Japanese patients with CML-CP or CML-AP. Our results also suggest that nilotinib may be useful for the treatment of patients with CML-BC or Ph+ ALL. Indeed, efficacy was observed in some CML-BC and Ph+ ALL patients; however, it remains to elucidate for which patient populations this drug would be most suitable in CML-BC and Ph+ ALL.

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