Comparison of Frequency and Sensitivity of *BCR-ABL1* Kinase Domain Mutations in Asian and White Patients With Imatinib-resistant Chronic–Phase Chronic Myeloid Leukemia

Hawk Kim,[1] SooHyun Kim,[2] Hyeoung-Joon Kim,[3] Yeo-Kyeoung Kim,[3] Jae-Yong Kwak,[4] Ho-Young Yhim,[4] Sung-Hyun Kim,[5] Young Rok Do,[6] Sukjoong Oh,[7] Sung-Eun Lee,[2,8] Saengsuree Jootar,[9] Jiu Wei Cui,[10] Dong-Wook Kim[2,11]

Abstract

Retrospective analysis of Asian and white patients with chronic myeloid leukemia (CML) was performed to assess the frequency of *BCR-ABL1* mutations in patients in whom frontline imatinib therapy had failed. Single mutations highly resistant to the second-generation tyrosine kinase inhibitors dasatinib, nilotinib, and bosutinib were found at a greater frequency in Asian than in white patients, stressing the importance of mutational analysis in optimizing CML therapy.

Introduction: *BCR-ABL1* mutations require consideration during second-line tyrosine kinase inhibitor selection for patients with chronic myeloid leukemia (CML). The present retrospective analysis compared the frequency of *BCR-ABL1* mutations in Asian and white patients in whom imatinib therapy had failed. **Patients and Methods:** A nonstudy cohort (76 Asian patients from community clinical practices) and 2 study cohorts (29 Asian and 352 white patients from dasatinib phase II and III clinical trials) were identified. **Results:** In the nonstudy cohort, 80 mutations were identified; the most frequent was T315I (15%), followed by phosphate-binding loop mutations E255K (11%), G250E (10%), and Y253H (10%). Asian patients had a greater proportion of T315I and phosphate-binding loop mutations compared with the white patients. The nonstudy cohort was less likely to have multiple mutations compared with either study cohort. Single mutations highly resistant to dasatinib, nilotinib, and bosutinib were more frequent in the Asian than in the white cohorts. **Conclusion:** These results suggest that mutational analysis findings will be invaluable for choosing an appropriate second-line tyrosine kinase inhibitor in Asia.

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Keywords: Asian countries, *BCR-ABL1* mutation, CML, Drug resistance, Imatinib

Introduction

Management of chronic myeloid leukemia (CML) was revolutionized by the advent of BCR-ABL1–targeted tyrosine kinase inhibitors (TKIs). Imatinib was the first approved TKI for the treatment of patients with newly diagnosed CML.[1,2] Although imatinib has demonstrated efficacy in these patients,

[1]Division of Hematology, Gachon University Gil Medical Center, Gachon University College of Medicine, Incheon, Korea
[2]Leukemia Research Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea
[3]Chonnam National University Hwasun Hospital, Hwasun-gun Jeollanam-do, Korea
[4]Chonbuk National University Hospital, Jeollabuk-do, Korea
[5]College of Medicine, Dong-A University, Busan, Korea
[6]School of Medicine, Keimyung University Dongsan Medical Center, Daegu, Korea
[7]Department of Hematology and Oncology, Kangbuk Samsung Hospital, School of Medicine, Sungkyunkwan University, Seoul, Korea
[8]Department of Hematology, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea
[9]Ramathibodi Hospital, Bangkok, Thailand
[10]The First Hospital of Jilin University, Changchun, China
[11]Catholic Hematology Hospital, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

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Address for correspondence: Dong-Wook Kim, MD, PhD, The Catholic University of Korea, 222 Banpodaero Seochogu, Seoul 06591, Korea
E-mail contact: dwkim@catholic.ac.kr

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approximately one third will develop resistance or will not respond to treatment.[3] Point mutations in the BCR-ABL1 kinase domain are recognized as the most frequent mechanism of resistance to imatinib.[1,4]

Point mutations within the *BCR-ABL1* kinase domain associated with imatinib resistance were first identified in patients with advanced disease who rapidly developed a relapse after an initial imatinib response.[5] These mutations facilitated reactivation of the BCR-ABL1 kinase, supporting their role in imatinib resistance. Subsequent studies reported that *BCR-ABL1* mutations occurred in 12% to 90% of patients with any phase CML who developed imatinib resistance.[4,6] *BCR-ABL1* mutations span the entire kinase domain, and > 90 have been identified to date.[7]

The BCR-ABL1 kinase domain can be divided into functional regions according to their role in the activation of BCR-ABL1.[8] A mutation at threonine 315, known as the "gatekeeper" position, confers the greatest resistance to imatinib and other BCR-ABL1 TKIs, including dasatinib, nilotinib, and bosutinib.[4,9-12] Ponatinib is the only TKI recommended for treatment of patients with this T315I mutation.[1] The threonine 315 position is critical for the normal functioning of the kinase and sits at the periphery of the adenosine triphosphate—binding site.[13] Moreover, the hydroxyl group on threonine 315 forms critical hydrogen bonds with imatinib, nilotinib, and dasatinib; thus, its mutation to isoleucine interferes with these drugs' ability to bind BCR-ABL1.[13]

Various studies established that mutations within the phosphate-binding loop (P-loop; residues 244-255) of the *BCR-ABL1* kinase domain are the most frequent point mutations in patients with imatinib resistance.[6,14-16] Both T315I and P-loop mutations have been associated with worse patient outcomes.[16-22] One study reported that all patients with a T315I mutation developed progression after a median of 13 months.[21] Another study reported that 92% of patients with P-loop mutations had died a median of 5 months after the mutation was detected compared with 21% of patients with non—P-loop mutations ($P = .0024$).[16] The presence of multiple mutations also can be predictive of poor outcomes.[23,24]

Not only can the results of mutational analysis identify the presence of mutations, they can also guide the management of CML after imatinib failure.[7,25] Dasatinib, nilotinib, and bosutinib are approved second-line therapies for patients with demonstrated resistance or intolerance to previous imatinib,[26-29] and each has a unique sensitivity profile with regard to *BCR-ABL1* mutations.[9-12,30-32] Although these TKIs are effective against most imatinib-resistant mutations, in vitro and clinical evidence has shown that several mutations are resistant to dasatinib (F317L/V/I/C, V299L, T315A, and T315I), nilotinib (Y253H, E255K/V, F359V/C/I, and T315I), and bosutinib (V299L and T315I). As discussed previously, ponatinib is the only TKI that has shown efficacy in patients with the T315I mutation.[33]

Information has been reported on *BCR-ABL1* kinase domain mutations in patients around the world[30-33]; however, this has usually been limited to specific Asian subpopulations.[34-39] It is also unclear whether the results obtained from clinical trials will translate into the community practice setting. We conducted a retrospective analysis to describe and compare the patterns of *BCR-ABL1* mutations in Asian patients from real-world clinical practices with those of Asian and white patients from multiple regions enrolled in dasatinib phase II and III clinical trials to further establish the most appropriate second-line TKI for patients with CML in whom imatinib has failed. Also, the frequency of the mutations in each cohort was compared after the mutations were separated by sensitivity to second-line TKIs based on in vitro 50% inhibitory concentration (IC_{50}) values. The present analysis provides a unique and comprehensive comparison of both real-world and study data for white and Asian patients.

Patients and Methods

Patients

From 2001 to 2013, 492 patients with imatinib-resistant chronic-phase CML (CML-CP) had ≥ 1 baseline mutation at the development of imatinib resistance. Of these, 416 patients were from 2 phase II clinical trials, CA180-013 [ClinicalTrials.gov identifier, NCT00101660; study of BMS-354825 (dasatinib) in patients with chronic myeloid leukemia who are either resistant or intolerant to imatinib][40] and CA180-017 [ClinicalTrials.gov identifier, NCT00103844; dasatinib (BMS-354835) versus imatinib mesylate in subjects with chronic myeloid leukemia],[41] and 1 phase III clinical trial, CA180-034 [ClinicalTrials.gov identifier, NCT00123474; chronic myelogenous leukemia (CML) - follow on: study of BMS-354825 in subjects with CML].[42] The patient characteristics and patient eligibility criteria have been previously described for these studies. Patients previously identified with 1 of the following mutations were excluded from CA180-017 enrollment: L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R.[41] No exclusions for previous mutation status were reported for CA180-013 or CA80-034. In the CA180-013 and CA180-017 trials, patients received dasatinib 70 mg twice daily (b.i.d.). In CA180-034, patients were randomized 1:1:1:1 to receive dasatinib 100 mg once daily, 50 mg b.i.d., 140 mg once daily, or 70 mg b.i.d. All institutional review boards and ethics committees approved these trials, and all patients gave written informed consent before randomization in accordance with the Declaration of Helsinki.

Of the 416 patients enrolled in the clinical trials, 381 were included in the present analysis. The race/ethnicity of the patients was collected during enrollment as part of the general demographic characteristics. The Asian study cohort consisted of 29 patients from 9 countries (Australia, Canada, Korea, Philippines, Singapore, Taiwan, Thailand, the United Kingdom, and the United States). The white study cohort consisted of 352 patients from 28 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Czechoslovakia, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Israel, Italy, Netherlands, Norway, Peru, Poland, Puerto Rico, Russia, South Africa, Spain, Sweden, Switzerland, the United Kingdom, and the United States). The remaining 35 patients were of other races and were not included in the present analysis.

Imatinib resistance was defined in CA180-013 as a lack of complete hematologic response (CHR) after 3 months of treatment, a lack of any cytogenetic response (CyR) after 6 months of treatment, a lack of a major cytogenetic response (MCyR; Philadelphia chromosome—positive cells > 35%) after 1 year of treatment, increased white blood cell count on ≥ 2 consecutive tests, or a relapse after either a CHR or an MCyR.[40] In CA180-017, primary imatinib resistance was defined as no CHR after 3 months, no CyR

Table 1 Baseline Patient Characteristics

Characteristic	Asian Nonstudy Cohort (n = 76)	Asian Study Cohort (n = 29)	White Study Cohort (n = 352)
Age, y			
Median	51	44	60
Range	18-83	24-68	15-85
Gender, n (%)			
Male	52 (68.4)	18 (62.1)	201 (57.1)
Female	24 (31.6)	11 (37.9)	151 (42.9)

after 6 months, no MCyR after 1 year, or a continuously increasing white blood cell count on 2 consecutive evaluations $\geq$ 2 weeks apart or an absolute increase in the white blood cell count $>$ 50,000/mm^3 greater than the nadir. Acquired imatinib resistance was defined as disease recurrence after a previous hematologic response or MCyR.[41] In CA180-034, primary imatinib resistance was defined as no decrease in the white blood cell count after $\geq$ 4 weeks of treatment, no CHR after 3 months, no MCyR after 6 months, and no complete CyR after 1 year. Acquired imatinib resistance was defined as the loss of MCyR ($\geq$ 30% absolute increase in Philadelphia chromosome–positive metaphases), loss of molecular response, evidence of a new mutation in the *BCR-ABL1* kinase domain, or loss of confirmed CHR.[42]

In addition, 76 patients with CML-CP from real-world clinical practices with $\geq$ 1 baseline mutation at the development of imatinib resistance were analyzed as part of the Asian nonstudy cohort (AMICA [patterns of ABL mutation in Asians with imatinib resistant chronic myeloid leukemia and Ph positive acute lymphocytic leukemia patients]; ClinicalTrials.gov identifier, NCT02034656) from 7 institutions from 3 Asian countries (China, South Korea, and Thailand).

Mutational Analysis

The frequencies of mutations and the proportions of single and multiple mutations were assessed in all 3 patient clinical trial cohorts. Samples for mutational assessment were collected before study treatment and once patients were withdrawn from the study, crossed over (CA180-017), or experienced disease progression (CA180-034). Mutational analysis was performed by a central laboratory for the dasatinib phase II/III studies.[40] Total RNA was extracted from mononuclear cells prepared from peripheral blood for mutation analysis; direct sequencing was performed as previously reported.[34] After nested polymerase chain reaction amplification, direct sequencing was performed to check the *BCR-ABL1* region corresponding to amino acids 237 to 486 in both the forward and the reverse direction using the ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA). Data from the Asian nonstudy cohort were collected from the patients' medical records and transcribed into a database for analysis. Mutational analyses were conducted by the patients' institute or tertiary institutes, including a commercial laboratory.

Comparison of BCR-ABL1 *Mutation Sensitivity to Second-line TKIs Determined by In Vitro IC_{50}-Fold Increase*

BCR-ABL1 mutation sensitivity to the second-line TKIs dasatinib, nilotinib, and bosutinib was assessed according to previously reported in vitro IC_{50}-fold increase results. Five sensitivity categories (sensitive, moderately resistant, resistant, highly resistant, unknown) based on the IC_{50}-fold increase greater than wild-type *BCR-ABL1* for each mutation were applied to the mutations for each TKI.[10]

Statistical Analysis

The study objectives were to compare the patterns and rates of *BCR-ABL1* mutations in Asian patients and white patients with imatinib-resistant CML-CP and to categorize the mutations by in vitro sensitivity to second-line TKIs. We also compared the frequency of the *BCR-ABL1* mutations that contributed to resistance to dasatinib, nilotinib, and bosutinib in Asian and white patients. The χ^2 test and Fisher exact test were used to test the correlation of the categorical variables. The 2-tailed Student *t* test was used to analyze continuous variables. All *P* values were 2-sided, and 5% was chosen as the level of statistical significance.

Results

Baseline Characteristics

A total of 457 patients with CML-CP with $\geq$ 1 mutation at imatinib failure were included in the present analysis (Asian nonstudy cohort, n = 76; Asian study cohort, n = 29, white study cohort, n = 352; Table 1). At baseline, patients in the Asian study cohort had the lowest median age (44 years; range, 24-68 years), followed by patients in the Asian nonstudy cohort (51 years; range, 18-83 years) and the white study cohort (60 years; range, 18-85 years). The proportion of male patients in both Asian cohorts was similar (68% and 62%) but was greater than that in the white study cohort (57%).

Frequency of BCR-ABL1 *Mutations*

In the Asian nonstudy cohort, 80 mutations were identified in the 76 patients assessed (Table 2). The most frequent *BCR-ABL1* mutation was T315I (15%). Collectively, P-loop mutations were most commonly identified (n = 38; 48%). The particular mutations within the P-loop of BCR-ABL1 that were frequently observed included E255K (11%), G250E (10%), and Y253H (10%).

In the dasatinib clinical trials, 34 individual mutations were identified in the 29 patients in the Asian study cohort, and 421 individual mutations were identified in the 352 patients from the white study cohort (Table 2). Fifteen P-loop mutations (44%) were found in the Asian study cohort, the most common of which was G250E (15%). In the white study cohort, 152 P-loop mutations (36%) were identified. The G250E mutation was also the most common in this group (12%).

Table 2 Frequency of Individual *BCR-ABL1* Mutations

Mutations	Asian Nonstudy and Study Cohorts (n = 105)	Asian Nonstudy Cohort (n = 76)	Asian Study Cohort (n = 29)	White Study Cohort (n = 352)	Asian and White Study Cohorts (n = 381)
Total	114	80	34	421	455
M244V	5 (4.4)	3 (3.8)	2 (5.9)	40 (9.5)	42 (9.2)
L248V	2 (1.8)	2 (2.5)	0 (0)	14 (3.3)	14 (3.1)
G250E	13 (11.4)	8 (10)	5 (14.7)	50 (11.9)	55 (12.1)
Q252H	6 (5.3)[a]	4 (5)[a]	2 (5.9)	5 (1.2)	7 (1.5)
Y253F	4 (3.5)[a]	3 (3.8)[a]	1 (2.9)	2 (0.5)	3 (0.7)[b]
Y253H	9 (7.9)	8 (10)	1 (2.9)	20 (4.8)	21 (4.6)
E255K	10 (8.8)[a]	9 (11.3)[c]	1 (2.9)	13 (3.1)	14 (3.1)[d]
E255V	4 (3.5)	1 (1.3)	3 (8.8)[a]	8 (1.9)	11 (2.4)
D276G	0 (0)	0 (0)	0 (0)	7 (1.7)	7 (1.5)
E279K	0 (0)	0 (0)	0 (0)	7 (1.7)	7 (1.5)
V299L	0 (0)	0 (0)	0 (0)	1 (0.2)	1 (0.2)
F311L	0 (0)	0 (0)	0 (0)	3 (0.7)	3 (0.7)
T315I	15 (13.2)[e]	12 (15)[e]	3 (8.8)	15 (3.6)	18 (4)[f]
T315A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
F317L	5 (4.4)	5 (6.3)	0 (0)	13 (3.1)	13 (2.9)
F317V	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M351T	6 (5.3)	2 (2.5)[a]	4 (11.8)	46 (10.9)	50 (11)[b]
F359V	6 (5.3)	4 (5)	2 (5.9)	25 (5.9)	27 (5.9)
V379I	1 (0.01)	1 (1.3)	0 (0)	2 (0.5)	2 (0.4)
L384M	0 (0)	0 (0)	0 (0)	2 (0.5)	2 (0.4)
L387M	2 (1.8)	2 (2.5)	0 (0)	4 (1)	4 (0.9)
H396R	2 (1.8)	1 (1.3)	1 (2.9)	28 (6.7)	29 (6.4)
H396P	2 (1.8)	2 (2.5)	0 (0)	4 (1)	4 (0.9)
F486S	1 (.01)	1 (1.3)	0 (0)	13 (3.1)	13 (2.9)
Other	21 (18.4)[g,h]	12 (15)[g]	9 (26.5)[h]	99 (23.5)[i]	108 (23.7)[h,i]

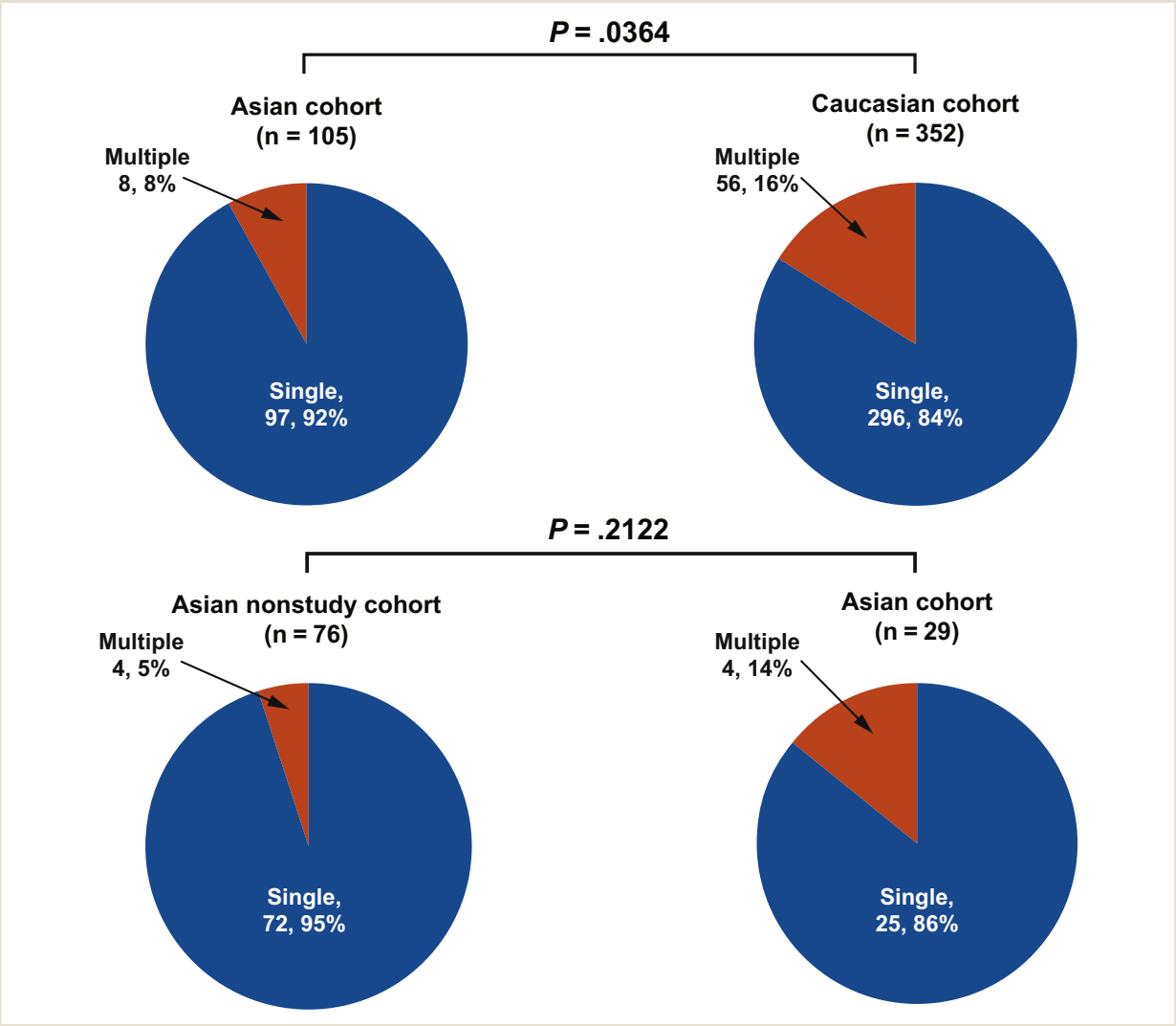
Data presented as n (%).
[a] $P < .05$ versus white study cohort.
[b] $P < .05$ versus Asian nonstudy cohort.
[c] $P < .01$ versus white study cohort.
[d] $P < .01$ versus Asian nonstudy cohort.
[e] $P < .001$ versus white study cohort.
[f] $P < .001$ versus Asian nonstudy cohort.
[g] Y215F, D287G, E281K, F311I, L323P, N336S, F359I, E450K (n = 2), E459K, W478R, P499L.
[h] L273M, E292V, F311I, E355G (n = 2), F359I, M388L, P480L (n = 2).
[i] H201L, M237V, I242T, K247R (n = 6), G250V, Y253K, E258D, L273M (n = 2), E281K, V289I, L298V, F311I (n = 3), F311V, Y342H, E355G (n = 16), F359C (n = 5), F359I (n = 11), D363Y, A365V, A366G, M388L (n = 2), Y393C, A397P (n = 2), S417Y, I418S, I418V (n = 3), S438C (n = 3), P441L, E450A (n = 2), E450G (n = 2), E450K (n = 2), E450V, E453K (n = 3), E453V, E459G, E459K (n = 11), M472I, G514S, del248-274 (n = 3).

Frequency of BCR-ABL1 *Mutations in Asian Versus White Cohorts*

The frequencies of *BCR-ABL1* mutations were compared by race among the Asian nonstudy cohort, the Asian study cohort, and the white study cohort (Table 2). In general, the proportion of P-loop mutations was greater in the Asian populations relative to the white study cohort. Specifically, the percentage of Q252H ($P < .05$), Y253F ($P < .05$), and E255K ($P < .01$) was greater in the Asian nonstudy cohort compared with the white study cohort, and the proportion of E255V ($P < .05$) was greater in the Asian study cohort than in the white study cohort. When the 2 Asian cohorts were combined, the proportion of Q252H ($P < .05$), Y253F ($P < .05$), and E255K ($P < .05$) was also greater than that in the white study cohort. The Asian populations also had a greater proportion of T315I mutations. The Asian nonstudy cohort ($P < .001$) and the combination of the 2 Asian populations ($P < .001$) had greater proportions of this mutation compared with the white cohort. In contrast, the proportion of M351T was lower in the Asian nonstudy cohort than in the white study cohort ($P < .05$) and was not different between the 2 study cohorts.

We also compared the mutation frequencies in the Asian nonstudy cohort to those in the Asian and white study cohorts. A greater percentage of T315I ($P < .001$) and P-loop mutations Y253F ($P < .05$) and E255K ($P < .01$) were reported in the Asian nonstudy cohort compared with the combination of the Asian and white study cohorts. The proportion of M351T was also lower in the Asian nonstudy cohort relative to the combination of the 2 study cohorts ($P < .05$). No statistically significant differences were found in the frequency of mutations between the Asian nonstudy cohort and the Asian study cohort.

Figure 1 Frequency of Patients With Single and Multiple *BCR-ABL1* Mutations. The Frequencies of Mutations and Proportions of Single or Multiple Mutations Were Assessed by Race. Blue, Single Mutations; Red, Multiple Mutations



Composition of BCR-ABL1 *Mutations*

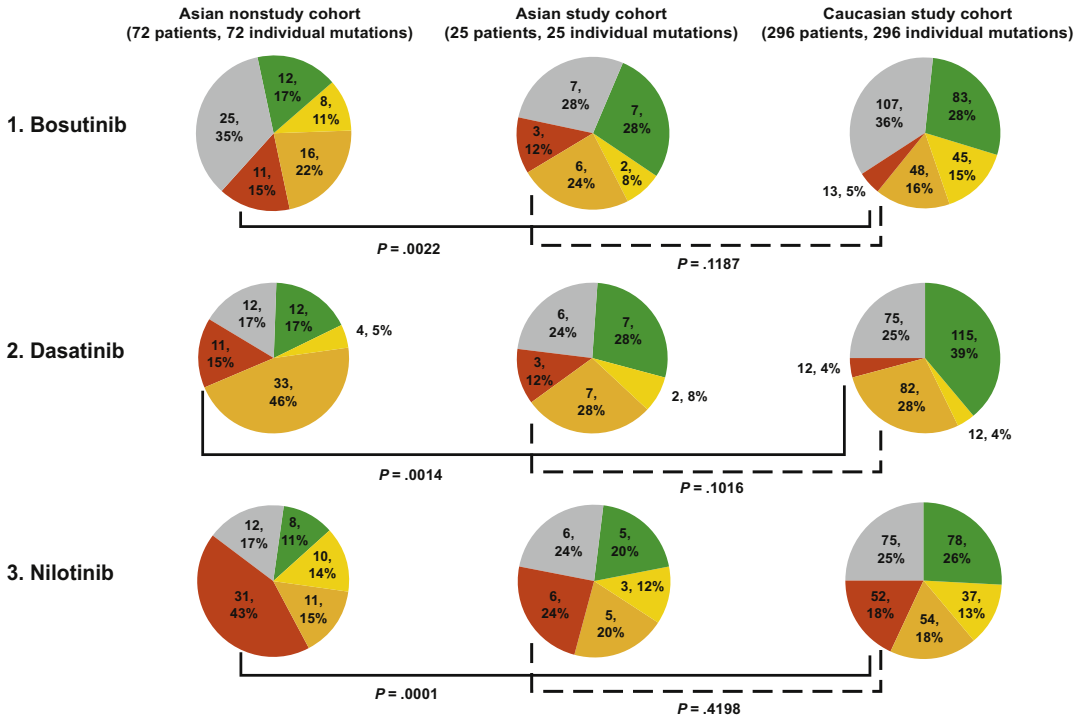
The proportion of patients with single and multiple mutations in the 3 cohorts was also assessed (Figure 1). A lower proportion of patients with mutations from the Asian nonstudy cohort had multiple mutations (5%) compared with both the Asian (14%) and the white study cohorts (16%). However, the difference between the Asian cohorts was not statistically significant ($P = .2122$). When the Asian cohorts were combined, the proportion of patients with multiple mutations (8%) was significantly greater in the white cohort (16%; $P = .0364$). The 2 study cohorts had similar proportions of patients with single and multiple mutations.

Frequency of Mutations by In Vitro Sensitivity to Second-line TKIs

Various studies of the in vitro sensitivity to TKIs, based on the IC_{50}-fold increase for many *BCR-ABL1* mutations, have been reported. These results have been used to help formulate the most recent European LeukemiaNet guidelines.[43] Mutations were considered sensitive to the TKI if the IC_{50}-fold increase greater than wild-type *BCR-ABL1* was ≤ 2, moderately resistant if > 2 to 4, resistant if > 4 to 10, and highly resistant if > 10. For the present analysis, only patients with a single mutation were included. When the mutations were divided according to their in vitro sensitivity to dasatinib, nilotinib, and bosutinib, a greater proportion of total mutations per cohort was highly resistant to all 3 TKIs in the Asian nonstudy cohort (total individual mutations, 72; dasatinib, 11; nilotinib, 31; bosutinib, 11) compared with the white study cohort (total individual mutations: 296; dasatinib, 12 [$P = .0014$]; nilotinib, 54 [$P = .0001$]; bosutinib, 13 [$P = .0022$]; Figure 2). This increase in highly resistant mutations was not seen in the Asian study cohort (total individual mutations: 25; dasatinib, 3; nilotinib, 6; bosutinib, 3) compared with the white study cohort (dasatinib, $P = .1016$; nilotinib, $P = .4198$; bosutinib, $P = .1187$). In the patients from the white study cohort, a greater proportion of

Figure 2 Frequency of *BCR-ABL1* Mutations by In Vitro Sensitivity. (A) Mutations Occurring in the Study Populations Were Analyzed by the Degree to Which They Contributed to Resistance To (1) Bosutinib, (2) Dasatinib, and (3) Nilotinib in Each Cohort. (B) Comparison of Mutations by In Vitro Sensitivity and Race. Only Patients With a Single Mutation Were Included. Green, Sensitive (50% Inhibitory Concentration [IC_{50}]-Fold Increase ≤ 2); Yellow, Moderately Resistant (IC_{50}-Fold Increase > 2-4); Orange, Resistant (IC_{50}-Fold Increase > 4-10); Red, Highly Resistant (IC_{50}-Fold Increase > 10); Gray, Unknown

A



B

Bosutinib	IC_{50} fold	G1 Asian nonstudy cohort (72 patients, 72 individual mutations)	G2 Asian study cohort (25 patients, 25 individual mutations)	G3 Caucasian study cohort (296 patients, 296 individual mutations)	*P*-value G1 vs. G2	*P*-value G2 vs. G3	*P*-value G1 vs. G3
Sensitive	≤ 2	12	7	83	.2479	1.0000	.0515
Moderately resistant	2.01-4	8	2	45	1.0000	.5536	.4565
Resistant	4.01-10	16	6	48	1.0000	.4001	.2286
Highly resistant	>10	11	3	13	1.0000	.1187	.0022
Unknown		25	7	107	.6265	.5163	.8914

Dasatinib	IC_{50} fold	G1 Asian nonstudy cohort (72 patients, 72 individual mutations)	G2 Asian study cohort (25 patients, 25 individual mutations)	G3 Caucasian study cohort (296 patients, 296 individual mutations)	*P*-value G1 vs. G2	*P*-value G2 vs. G3	*P*-value G1 vs. G3
Sensitive	≤ 2	12	7	115	.2479	.3912	.0003
Moderately resistant	2.01-4	4	2	12	.6460	.2987	.5283
Resistant	4.01-10	33	7	82	.1583	1.0000	.0043
Highly resistant	>10	11	3	12	1.0000	.1016	.0014
Unknown		12	6	75	.5506	1.0000	.1631

Nilotinib	IC_{50} fold	G1 Asian nonstudy cohort (72 patients, 72 individual mutations)	G2 Asian study cohort (25 patients, 25 individual mutations)	G3 Caucasian study cohort (296 patients, 296 individual mutations)	*P*-value G1 vs. G2	*P*-value G2 vs. G3	*P*-value G1 vs. G3
Sensitive	≤ 2	8	5	78	.3099	.6361	.0051
Moderately resistant	2.01-4	10	3	37	1.0000	1.0000	.6986
Resistant	4.01-10	11	5	54	.5493	.7905	.6096
Highly resistant	>10	31	6	52	.1014	.4198	.0001
Unknown		12	6	75	.5506	1.0000	.1631

mutations was resistant to dasatinib compared with those in the Asian nonstudy cohort ($P = .0043$), and more mutations were sensitive to dasatinib and nilotinib compared with those in the Asian nonstudy cohort ($P = .0003$ and $P = .0051$, respectively; Figure 2B).

Discussion

Several studies have reported the frequency of *BCR-ABL1* mutations in patients with CML enrolled in international clinical trials.[30-33] However, the *BCR-ABL1* mutation frequency has only been reported for a limited number of Asian patients.[34-39] For clinical trials initiated after 2005, investigators became aware that some mutations, including T315I and P-loop mutations, could be resistant to dasatinib, nilotinib, and bosutinib.[9-12,43] Consequently, patients with these mutations might have been excluded by investigators, resulting in an underestimation of mutation frequency in these patients. Therefore, to determine the effect of this potential exclusion and the influence of the race on *BCR-ABL1* mutation frequency, we collected mutation data from Asian patients resistant to imatinib in real-world clinical practice and compared them with the mutation analysis from imatinib-resistant Asian and white patients enrolled in dasatinib phase II/III clinical trials. The goal of our assessment was to provide clinicians in Asian countries with evidence to guide treatment selection after imatinib resistance.

Similar to previous studies of Asian patients with CML resistant to imatinib,[34-36] the most common mutations identified in the Asian nonstudy cohort were T315I and P-loop mutations. More *BCR-ABL1* mutations identified in the Asian nonstudy cohort were T315I or within the P-loop compared with the study cohorts analyzed in the present study. Specifically, 15% of the mutations in the Asian nonstudy cohort were T315I, 5% were Q252H, and 11% were E225K compared with 4%, 2%, and 3%, respectively, for the combined study cohorts. This likely reflects a lack of patient enrollment with these mutations in the dasatinib clinical trials, owing to the known unresponsiveness of T315I and some P-loop mutations to dasatinib.[10,31,43] However, when a separate mutational analysis was conducted by race, Asian patients had a greater proportion of T315I and certain P-loop mutations. Even within the study cohorts, Asian patients had a greater proportion of T315I (9%) and E255V (9%) compared with white patients (4% and 2%, respectively). Thus, these mutations might be more prevalent in Asian populations, suggesting that Asian patients with CML with imatinib resistance might have a worse prognosis than their white counterparts.

We also found a difference in the proportion of patients with multiple mutations in the Asian nonstudy cohort (5%) compared with the study cohorts (white, 16%; Asian, 14%). In contrast, a greater proportion of Asian versus white patients had single mutations highly resistant to each of the second-line TKIs. Although the present study did not determine whether patients with multiple mutations had compound or polyclonal mutations, it has been reported that 70% of patients with multiple mutations have compound mutations.[44] Compound mutations, or mutations within the same allele of *BCR-ABL1*, can confer a high level of resistance to TKI treatment.[44]

A comparison of mutations identified in the Asian nonstudy cohort, the Asian study cohort, and the white study cohort by in vitro sensitivity to TKIs was also performed. In both Asian cohorts, more mutations were classified as highly resistant (IC_{50}-fold increase > 10) to dasatinib, nilotinib, or bosutinib compared with the white cohort. Across all cohorts analyzed, a greater proportion of mutations sensitive to dasatinib or bosutinib were identified compared with nilotinib-sensitive mutations. Although a more formal efficacy analysis is required to draw definitive conclusions, these data on the frequency of TKI-specific resistant mutations suggest that Asian and white patients might be more likely to respond to treatment with dasatinib or bosutinib versus nilotinib.

A significantly ($P < .05$) greater proportion of all Asian versus white patients developed the clinically relevant mutation T315I (13.4% vs. 3.6%), which is resistant to imatinib, dasatinib, nilotinib, and bosutinib. The same trend was observed for mutations identified in patients with CML as clinically resistant to nilotinib and bosutinib (E255K, 7% vs. 3.1%). These results suggest that mutational analysis is especially important in Asian populations when choosing a second-line TKI after imatinib failure.

The reason Asian patients experienced a greater frequency of resistant mutations compared with white patients is unclear and requires further investigation to discern the cause. Older age, previous interferon-α treatment,[45] and higher imatinib dose[6] have all been reported as potential causes of increased mutation development. In the present study, Asian patients had a lower median age than white patients, suggesting that age was likely not a factor. In the 3 clinical trials used for our analysis, most of the total study populations (52%-71%) had previously been treated with interferon-α at some point, implying that an overall increase would occur in mutation frequency versus an increase solely in Asian patients. Finally, if the study groups were all receiving the same dose, because Asian patients tend to have a smaller body size compared with non-Asian patients,[46] the dose-to-weight ratio would be greater in Asian patients and potentially increase the risk of mutation incidence by early selection of minor mutation clones. Additional analyses are required to determine whether any specific causes result in the increased mutation incidence in the Asian population.

The present retrospective analysis had limitations. First, differences could have been present in the definition of imatinib failure and the imatinib dose between the Asian nonstudy cohort and the study cohorts. Also, patients from the Asian nonstudy cohort had mutational analyses conducted by the patients' institute or tertiary institutes, including a commercial laboratory. In contrast, the patients enrolled in the clinical trials had mutational analyses performed by a central laboratory. Additionally, limited numbers of Asian patients were available for inclusion in both the nonstudy and the study cohort compared with white patients.

Conclusion

Asian patients with *BCR-ABL1* mutations, both within clinical trials and in the community setting, had a greater proportion of T315I and P-loop mutations compared with the white patients. Asian patients also had a greater proportion of mutations considered highly resistant to TKIs compared with the white cohort based on in vitro studies. These results suggest that specific actions are required to optimize CML management in Asian countries. First, mutational analysis is a valuable strategy to determine the optimal second-line TKI therapy best suited for individual patients,

especially owing to the frequency of highly resistant mutations identified in Asian patients. Improving mutational analysis procedures and encouraging the use of mutational analysis will be critical to the successful management of CML in Asian countries. Future regional studies are warranted to confirm whether differences exist in the pharmacokinetic responses of patients with CML to various TKIs, including ponatinib (now approved for use in the second line), which could help determine the optimal dosage of TKIs for patients of Asian versus non-Asian descent.

Clinical Practice Points

- Resistance to frontline imatinib therapy for the treatment of CML is often due to the development of imatinib-resistant mutations in the *BCR-ABL1* gene, requiring changes to the TKI treatment.
- *BCR-ABL1* mutations have been reported to negatively affect patient outcomes; specifically, the T315I mutation has been linked to progression and patients with mutations in the P-loop (residues 244-255) might have lower survival rates than patients with non−P-loop mutations.
- Each BCR-ABL1 TKI has its own unique profile regarding sensitivity to mutations; therefore, obtaining information on a patient's *BCR-ABL1* profile would be useful in guiding and optimizing treatment of CML.
- Our analysis of Asian and white patients with CML found that Asian patients had a greater proportion of highly resistant mutations, P-loop mutations, and the T315I mutation compared with white patients.
- These findings suggest that performing mutational analysis of Asian patients, when designing their CML treatment plan and determining second-line therapy after imatinib, can be beneficial in selecting the most appropriate and effective TKI based on *BCR-ABL1* mutation sensitivity.

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References

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Chronic Myelogenous Leukemia, version 1. 2016, Available at: https://www.nccn.org/professionals/physician_gls/pdf/cml.pdf. Accessed: August 31, 2016.
2. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood* 2006; 108:1809-20.
3. Bixby D, Talpaz M. Seeking the causes and solutions to imatinib-resistance in chronic myeloid leukemia. *Leukemia* 2011; 25:7-22.
4. Soverini S, Branford S, Nicolini FE, et al. Implications of BCR-ABL1 kinase domain-mediated resistance in chronic myeloid leukemia. *Leuk Res* 2014; 38: 10-20.
5. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 2001; 293: 876-80.
6. Jabbour E, Kantarjian H, Jones D, et al. Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia* 2006; 20:1767-73.
7. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. *Blood* 2011; 118:1208-15.
8. Schindler T, Bornmann W, Pellicena P, et al. Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. *Science* 2000; 289:1938-42.
9. O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res* 2005; 65:4500-5.
10. Redaelli W, Piazza R, Rostagno R, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol* 2009; 27: 469-71.
11. Weisberg E, Manley P, Mestan J, et al. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer* 2006; 94:1765-9.
12. Bradeen HA, Eide CA, O'Hare T, et al. Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. *Blood* 2006; 108:2332-8.
13. Gibbons DL, Pricl S, Kantarjian H, et al. The rise and fall of gatekeeper mutations? The BCR-ABL1 T315I paradigm. *Cancer* 2012; 118:293-9.
14. Corbin AS, La Rosee P, Stoffregen EP, et al. Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. *Blood* 2003; 101:4611-4.
15. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood* 2005; 105:2640-53.
16. Branford S, Rudzki Z, Walsh S, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood* 2003; 102:276-83.
17. Soverini S, Martinelli G, Rosti G, et al. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. *J Clin Oncol* 2005; 23:4100-9.
18. Khorashad JS, de Lavallade H, Apperley JF, et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. *J Clin Oncol* 2008; 26:4806-13.
19. Soverini S, Colarossi S, Gnani A, et al. GIMEMA Working Party on Chronic Myeloid Leukemia. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin Cancer Res* 2006; 12:7374-9.
20. Nicolini FE, Corm S, Le QH, et al. Mutation status and clinical outcome of 89 imatinib mesylate-resistant chronic myelogenous leukemia patients: a retrospective analysis from the French intergroup of CML (Fi(phi)-LMC GROUP). *Leukemia* 2006; 20:1061-6.
21. Nicolini FE, Hayette S, Corm S, et al. Clinical outcome of 27 imatinib mesylate-resistant chronic myelogenous leukemia patients harboring a T131I BCR-ABL mutation. *Haematologica* 2007; 92:1238-41.
22. Jabbour E, Kantarjian H, Jones D, et al. Characteristics and outcomes of patients with chronic myeloid leukemia and T315I mutation following failure of imatinib mesylate therapy. *Blood* 2008; 112:53-5.
23. Quintas-Cardama A, Kantarjian H, O'Brien S, et al. Pleural effusion in patients with chronic myelogenous leukemia treated with dasatinib after imatinib failure. *J Clin Oncol* 2007; 25:3908-14.
24. Parker WT, Ho M, Scott HS, et al. Poor response to second-line kinase inhibitors in chronic myeloid leukemia patients with multiple low-level mutations, irrespective of their resistance profile. *Blood* 2012; 119:2234-8.
25. *Sprycel® (dasatinib) [Prescribing Information]*. Princeton, NJ: Bristol-Myers Squibb Co; 2015.
26. *Tasigna® (nilotinib) [Prescribing Information]*. East Hanover, NJ: Novartis Pharmaceutical Corporation; 2015.
27. *Gleevec® (imatinib) [Prescribing Information]*. East Hanover, NJ: Novartis Pharmaceutical Corporation; 2015.
28. *Bosulif® (bosutinib) [Prescribing Information]*. New York, NY: Pfizer Inc; 2015.
29. Hughes T, Saglio G, Branford S, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. *J Clin Oncol* 2009; 27:4204-10.
30. Müller MC, Cortes JE, Kim DW, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: analysis of responses according to preexisting BCR-ABL mutations. *Blood* 2009; 114:4944-53.

31. Khoury HJ, Cortes JE, Kantarjian HM, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. *Blood* 2012; 119:3403-12.
32. Shah NP, Skaggs BJ, Branford S, et al. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J Clin Invest* 2007; 117:2562-9.
33. Hochhaus A, Kim DW, Pinilla-Ibarz J, et al. Molecular responses with ponatinib in patients with Philadelphia chromosome positive (Ph+) leukemia: results from the PACE trial. *Blood* 2012; 120:3763.
34. Kim SH, Kim D, Kim DW, et al. Analysis of Bcr-Abl kinase domain mutations in Korean chronic myeloid leukaemia patients: poor clinical outcome of P-loop and T315I mutation is disease phase dependent. *Hematol Oncol* 2009; 27:190-7.
35. Kagita S, Uppalapati S, Jiwatani S, et al. Incidence of Bcr-Abl kinase domain mutations in imatinib refractory chronic myeloid leukemia patients from South India. *Tumour Biol* 2014; 35:7187-93.
36. Elias MH, Baba AA, Azlan H, et al. BCR-ABL kinase domain mutations, including 2 novel mutations in imatinib resistant Malaysian chronic myeloid leukemia patients—frequency and clinical outcome. *Leuk Res* 2014; 38:454-9.
37. Qin Y, Chen S, Jiang B, et al. Characteristics of BCR-ABL kinase domain point mutations in Chinese imatinib-resistant chronic myeloid leukemia patients. *Ann Hematol* 2011; 90:47-52.
38. Itonaga H, Tsushima H, Imanishi D, et al. Molecular analysis of the BCR-ABL1 kinase domain in chronic-phase chronic myelogenous leukemia treated with tyrosine kinase inhibitors in practice: study by the Nagasaki CML Study Group. *Leuk Res* 2014; 38:76-83.
39. Markose P, Chendamarai E, Balasubramanian P, et al. Spectrum of BCR-ABL kinase domain mutations in patients with chronic myeloid leukemia from India with suspected resistance to imatinib-mutations are rare and have different distributions. *Leuk Lymphoma* 2009; 50:2092-5.
40. Hochhaus A, Kantarjian HM, Baccarani M, et al. Dasatinib induces notable hematologic and cytogenetic responses in chronic-phase chronic myeloid leukemia after failure of imatinib therapy. *Blood* 2007; 109:2303-9.
41. Kantarjian H, Pasquini R, Lévy V, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia resistant to imatinib at a dose of 400 to 600 milligrams daily. *Cancer* 2009; 115:4136-47.
42. Shah NP, Kantarjian HM, Kim DW, et al. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. *J Clin Oncol* 2008; 26:3204-12.
43. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood* 2013; 122:872-84.
44. Khorashad JS, Kelley TW, Szankasi P, et al. BCR-ABL1 compound mutations in tyrosine kinase inhibitor-resistant CML: frequency and clonal relationships. *Blood* 2013; 121:489-98.
45. Ramirez P, DiPersio JF. Therapy options in imatinib failures. *Oncologist* 2008; 13: 424-34.
46. Kim D-W, Goh Y-T, Hsiao H-H, et al. Clinical profile of dasatinib in Asian and non-Asian patients with chronic myeloid leukemia. *Int J Hematol* 2009; 89:664-72.