SciMedCentral

Journal of Hematology & Transfusion

Review Article

BCR-ABL Point Mutations and TKI Treatment in CML Patients

Shinya Kimura*, Toshihiko Ando and Kensuke Kojima

Department of Internal Medicine, Division of Hematology, Saga University, Japan

Abstract

Most newly diagnosed CML patients in the Chronic Phase (CP), when treated with imatinib, achieve durable responses. However, a small percentage of these patients as well as most advanced-phase patients relapse on imatinib therapy. Among several resistant mechanisms, "point mutation within the BCR-ABL kinase domain" that interferes with imatinib binding is most important. To overcome imatinib-resistance, four second generation ABL Tyrosine Kinase Inhibitors (TKIs) such as dasatinib, nilotinib, bosutinib and bafetinib were developed. Since there are slight differences in the strong and weak points for each mutation among each TKI, it is very important to identify the type of *bcr-abl* mutationpresent in ABL TKI-resistant patients. Unfortunately, none of second generation BCR-ABL TKIs can inhibit T315I clone. Thus, a third-generation BCR-ABL TKI, ponatinib was developed and had been already used in clinic. Furthermore, a lot of novel agents which can override BCR-ABL KD mutations including T315I are being developed.

Special Issue on

Future Aspects of Chronic Myeloid Leukemia Treatment

***Corresponding author**

Shinya Kimura, Department of Internal Medicine, Division of Hematology, Respiratory Medicine and Oncology, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan, Tel: +81-952-34-2353; Fax: +81-952-34-2017; Email: shkimu@cc.saga-u.ac.jp

Submitted: 25 May 2014

Accepted: 27 June 2014

Published: 08 July 2014

ISSN: 2333-6684

Copyright

© 2014 Kimura et al.

OPEN ACCESS

Keywords

- Chronic myeloid leukemia
- bcr-abl
- Mutation
- Tyrosine kinase inhibitor

ABBREVIATIONS

Ph: Philadelphia; CML: Chronic Myeloid Leukemia; ALL: Acute Lymphoblastic Leukemia; TK: Tyrosine Kinase; CP: Chronic Phase; KD: Kinase Domain; TKI: Tyrosine Kinase Inhibitor; SNP: Single Nucleotide Polymorphism; A-loop: Activation-Loop; P-loop: Phosphate Binding-Loop; C-loop: Catalytic-Loop; DS: Direct Sequencing; ASO: Allele-Specific Oligonucleotide; PNA: Peptide Nucleic Acid; QP: Guanine-Quenching Probes; MBP: Mutation-Biased PCR; UDS: Ultradeep Sequencing; SFKs: SRC Family Kinases; FDA: Food and Drug Administration; HHT: Homoharringtonine

INTRODUCTION

Philadelphia (Ph) chromosome results from a reciprocal translocation between chromosomes 9 and 22 and generates the fusion *bcr-abl* chimericgene (Figure 1A), the cause of Chronic Myeloid Leukemia (CML) and Ph positive acute lymphoblastic leukemia (Ph^+ALL) [1]. Chemotherapy and interferon-αcould not prolong significantly the survival of CML and Ph^+ALL patients. Imatinib mesylate (Gleevec™, Glivec™) specifically inhibits the autophosphorylation of ABL Tyrosine Kinase (TK) (Figure 1B) and is not only highly efficacious in treating these diseases but also generally produces only mild side effects [2]. Within a few years of its introduction to the clinic, imatinib dramatically altered the first line therapy for CML and prolonged survival of CML patient (Figure 2) [3]. Most newly diagnosed CML patients in the Chronic Phase (CP), when treated with imatinib, achieve durable responses. However,a small percentage of these patients as well as most advanced-phase patients relapse on imatinib therapy [4,5]. Among several resistant mechanisms, "point mutation within the BCR-ABL Kinase Domain (KD)"that interferes with imatinib binding is most important. Currently, more than 100 mutations were found in imatinib-resistant CML patients [6-9].

To overcome imatinib-resistance, four second generation ABL Tyrosine Kinase Inhibitors (TKIs) (dasatinib (Sprycel™) [10], nilotinib (Tasigna™)[11], bosutinib (Bosulif™) [12] and bafetinib [13]) were developed.Since there are slight differences in the strong and weak points for each mutation among each TKI, [14-16], it is very important to identify the type of *bcr-abl* mutationpresent in ABL TKI-resistant patients.

Unfortunately, none of second generation BCR-ABL TKIs can inhibit a point mutation of threonine 315 to isoleucine (T315I).Thus, a third-generation BCR-ABL TKI ponatinib was developed and had been already used in clinic [17]. Furthermore, a lot of novel agents which can override BCR-ABL KD mutations including T315I are being developed. This review will focus on the mechanisms of resistance by mutations, methods of mutation detection and novel agents which are developed to override mutations.

Imatinib and mutations

Mechanisms of imatinib resistance: According to the results in the 8th year of the IRIS study, the administration of imatinib could not be continued over a long period in about one third of patients with CML-CP due to resistance or intolerance [18]. As causes of imatinib-resistance, point mutation of the ATP-binding region in the BCR-ABL KD, which is the binding site of imatinib

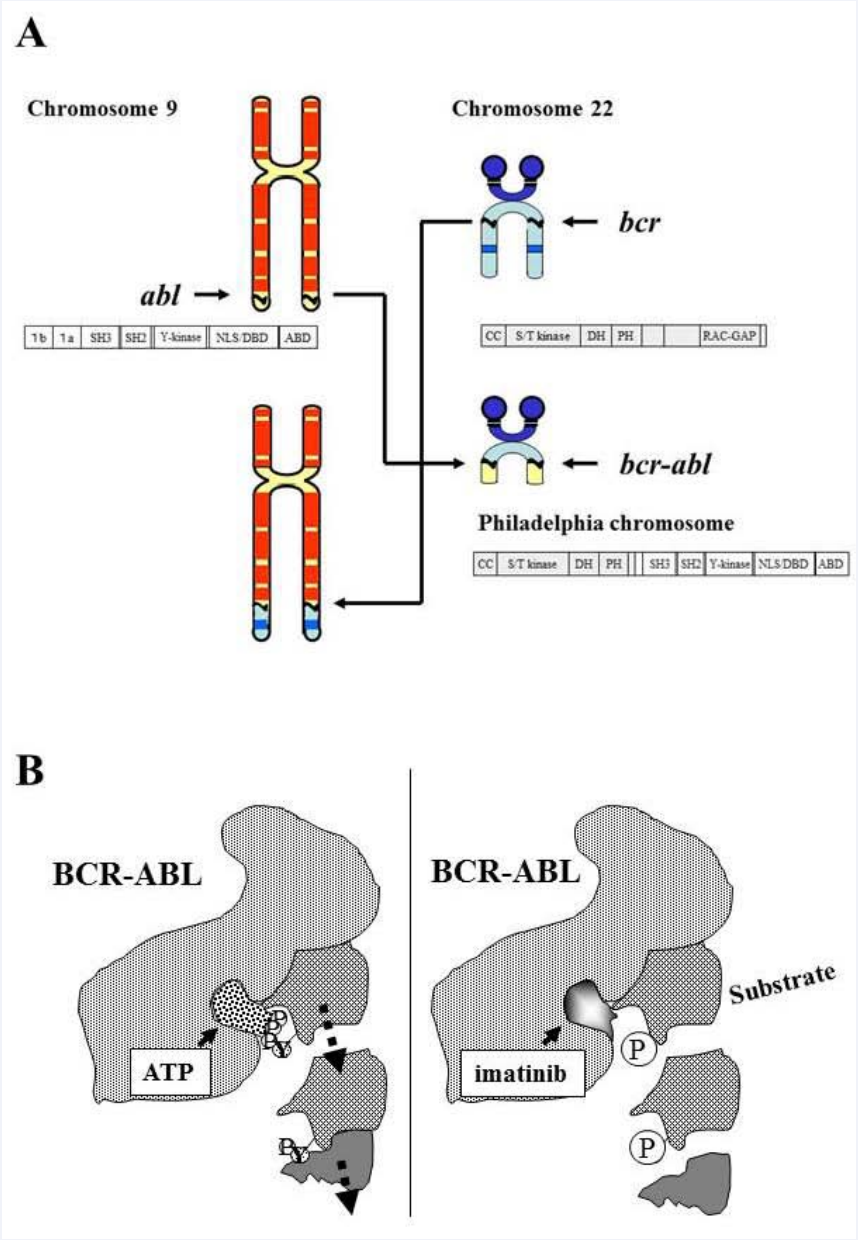

Figure 1 Philadelphia chromosome and the action of imatinib.

(A) Reciprocal translocation between chromosome 9 and chromosome 22 forms an extra-long chromosome 9 and the Philadelphia chromosome containing the fused *bcr-abl* gene. Fused *bcr-abl* gene is translated to BCR-ABL chimera protein that causes CML and Ph⁺ALL, (B) The BCR-ABL tyrosine kinase (TK) is a constitutively active kinase that functions by binding ATP and transferring phosphate from ATP to tyrosine residues on various substrates. This causes excess proliferation of leukemia cells characteristic of CML and Ph⁺ALL. Imatinib functions by blocking the binding of ATP to the BCR-ABL TK, inhibiting its activity. In the absence of TK activity, substrates required for BCR-ABL function cannot be phosphorylated, and subsequent cellular events are abrogated.

[6], amplification of the *bcr-abl* gene and an increase in the number of mRNA copies [6],emergence of multi-drug resistance genes [19], activation of other oncogenes (SRC, LYN, etc.)[20], and low expression of the drug transporter Oct-1 [21], have been reported. BIM which is a molecule for the induction of apoptosis, had already been reported to be important for the effects of imatinib [22], and it was recently reported that deletion-type polymorphism in an intron of a gene coding for BIM is involved in the poor response to TKIs [23]. Although French group did not found mutations with amino-acid change in the BIM coding sequence, observed a silent single nucleotide polymorphism (SNP) c465C>T (rs724710). T allele frequency was higher in non-responsive patients than in the reference population [24].

Imatinib-resistant mutations: Among above resistant mechanisms, point mutation within the BCR-ABL KD is most important. Indeed, 60~80 % of the cases of imatinib resistance are due to this point-mutation mechanism. Four main point

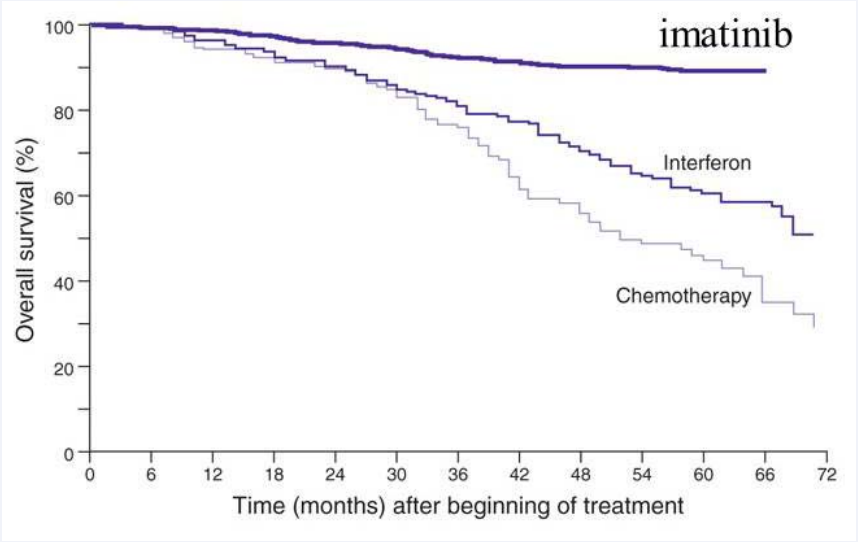

Figure 2 Survival of patients with CML.

Curves show overall survival for patients with CML treated with imatinib, interferon or conventional chemotherapy. Conventional chemotherapy delivers only a minimal effect on survival compared with no treatment [3].

mutations sites that result in imatinib resistance have been reported: i) the direct binding site, ii) the phosphate binding-loop (P-loop), iii) the activation-loop (A-loop) and iv) the catalytic-loop (C-loop) (Figure 3) [25]. i) The direct binding site T315 is essential for hydrogen bonding between imatinib and ABL; thus, a point mutation of threonine 315 to isoleucine (T315I) interferes with this hydrogen bond. Furthermore, the T315I mutation induces a conformational change in several amino acid residues, which are important for the binding of imatinib and BCR-ABL (the allosteric effect). Accordingly, T315I yields imatinib resistance more strongly than other point mutations (Figure 4)[25]. ii) P-loop is called the induced fit site because of its conformational change accompanied by imatinib binding [26]. This induced-fit enables imatinib to make a hydrogen bond with tyrosine 253 (Y253) that is intensified by other hydrophobic amino acids surrounding it. Therefore, the point mutations at Y253 including Y253F and Y253H interfere with imatinib binding to Y253. iii) Imatinib can only bind to inactive ABL which kinase-active site is closed by the activation-loop (A-loop); this loop is involved in ABL specificity and resistance.iv) Mutations within the C-loop such as M351T also induce a conformational change of active ABL, while mutations within the A-loop prompt ABL to form a more active conformation [25].

Among these mutations, Y253H, E255K, T315I, and M315T are observed more frequently. Also, multiple mutations causing imatinib resistance may be detected simultaneously. When multiple mutations are detected, mutations may be present in different BCR-ABL molecules (polyclonal mutation) or in a single BCR-ABL molecule (compound mutation) (Figure 5). Compound mutations have been reported to often cause stronger resistance to TKIs [27].

Other than the ATP-binding site, mutation of the SH3-SH2 domain of BCR-ABL (T212R) has been reported to be involved in resistance [28]. Alternatively spliced BCR-ABL mRNA with a 35-bp insertion (35INS) between ABL kinase domain exons 8 was also reported in imatinib-resistant patients which insertion resulted in a frame shift leading to the addition of 10 residues and truncation of 653 residues due to early termination [29]. However, it was also reported that this 35INS insertion/truncation mutant is kinase-inactive and does not contribute to tyrosine kinase inhibitor resistance in CML [30].

Pre-existing mutation: BCR-ABL KD mutations can exist in the newly diagnosed CML-CP patients and may affect the outcome of imatinib treatment. There are limited data available from imatinib-naive patients in CML-CP regarding the incidence of BCR-ABL KD mutations, and the correlation of these mutations with the therapeutic response in unselected patients has not been established [31]. To clarify the meaning of pre-existing mutaions in CML patients, the examination of mutation on CML stem cell may be useful. Previous studies indicated that a small population of $CD34^+$ CML (stem/progenitor) cells are less responsive to imatinib and other TKIs, and act as a reservoir for the emergence of imatinib-resistant subclones [32]. Pre-existing ABL kinase domain mutations in $CD34^+$ cells examined by multiplex allele-specific PCR were detected in 32/100 patients and included F311L, M351T, and T315I and all patients with pre-existing BCR-ABL mutations exhibited imatinib resistance [33].

Methods for detection ofBCR-ABL KD mutation

As mentioned above, mutation testing should be performed to ensure the successful therapeutic management of CML and Ph^+ALL patients when they showed resistance to imatinib. Various techniques have been developed to detect mutations. Direct Sequencing (DS) is widely used but is cumbersome, time-consuming and of low sensitivity which may be insufficient for optimal treatment of CML and Ph^+ALL patients (detection limit is around 25%) [34]. Several alternative methods have been reported, such as D-HPLC [35], allele-specific oligonucleotide PCR (ASO-PCR) [36], pyrosequencing [37], peptide nucleic acid (PNA)-based PCR clamping [38] and four-channel asymmetric

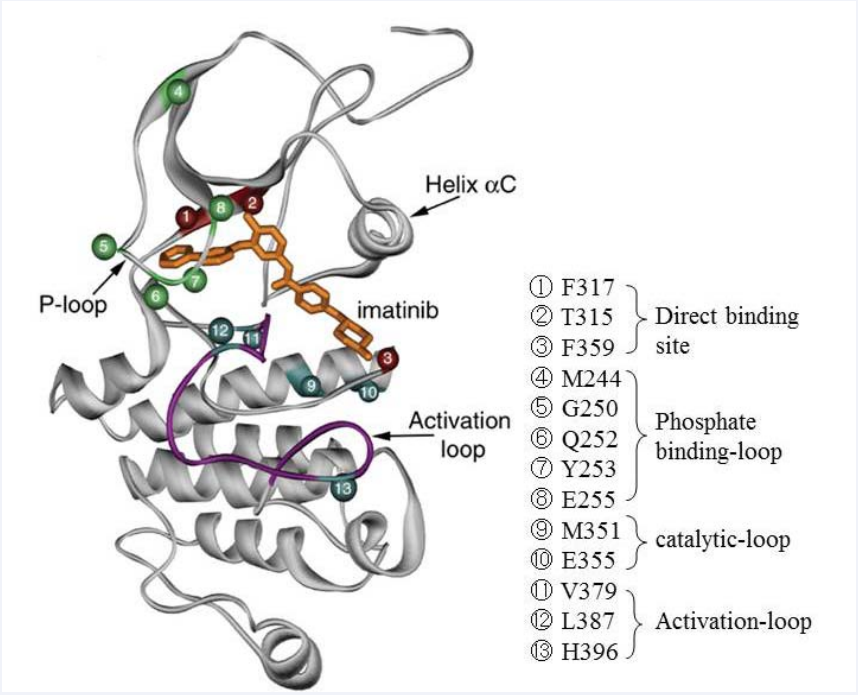

Figure 3 Proposed mechanisms of action of imatinib-resistant mutations based upon the crystal structure of ABL complexed with imatinib.

Ribbon representation of the kinase domain of ABL complexed to imatinib, depicting resistant mutations. Imatinib is shown in gold. Positions 1–3 (red) are mutations that directly affect imatinib binding. All other positions are those that likely affect the ability of the kinase to achieve the conformation required to bind imatinib, including those in the P loop (4–8; green) and those in the vicinity of the activation loop (9–13; cyan). The activation loop is colored purple. The positions of amino acids found mutated are depicted by spheres: 1, F317; 2, T315; 3, F359; 4, M244; 5, G250; 6, Q252; 7, Y253; 8, E255; 9, M351; 10, E355; 11, V379; 12, L387; 13, H396 [25].

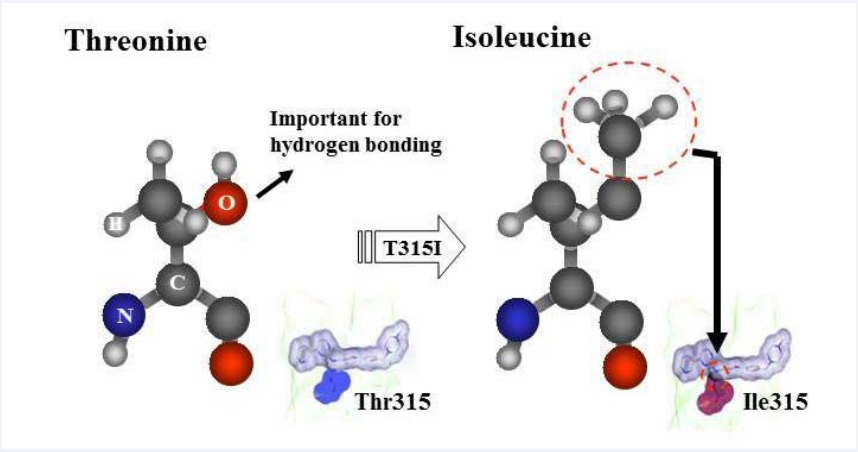

Figure 4 The structures of threonine and isoleucine.

When the amino acid (aa) at 315 is substituted to isoleucine from threonine, hydrogen bonding between threonine at aa315 and imatinib is lost. Moreover, the bigger side chain of isoleucine (red dotted circle) becomes an obstacle for the binding of imatinib to the ATP binding pocket.

real-time PCR hybridization probe assay[39]. Although these methods are more sensitive than the conventional DS, they are not easily automated. Thus, we developed an automatic method utilizing guanine-Quenching Probes (QP) to detect 17 kinds of mutations frequently observed in imatinib-resistance. Results were obtained from 100 μL of whole blood within 90 minutes by this method with high sensitivity at 3%. Furthermore, we developed a more sensitive method, mutation-biased PCR-QP (MBP-QP), which is 10 times more sensitive than the original QP method, in order to specifically detect the T315I clone [40].

Recently, ultradeep sequencing (UDS) was used to quantitatively and qualitatively evaluate mutations in patients with CML and Ph^+ALL [41]. Compared with conventional DS, UDS detected additional "minor" mutations in 55% of samples tested. Additionally, UDS was able to uncover a high degree of complexity

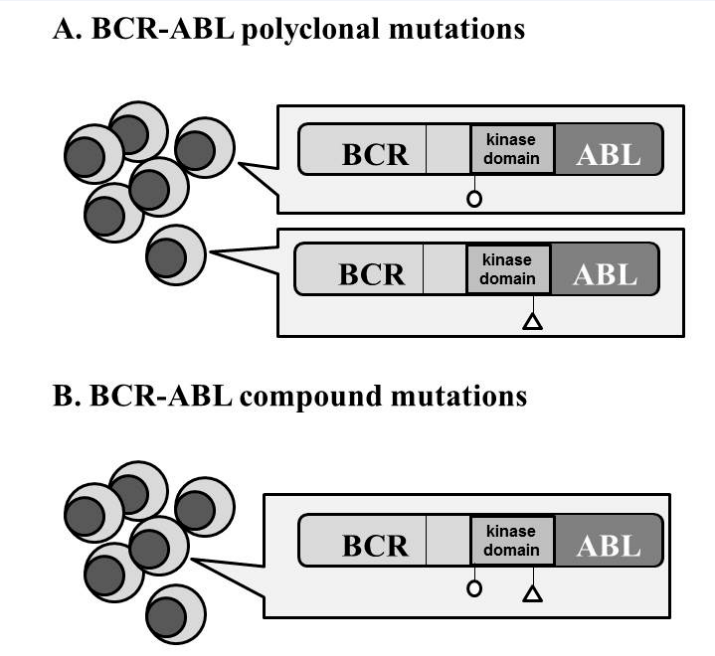

Figure 5 Polyclonal versus compound mutations.

In a subset of patients who develop clinical resistance to ABL TKIs, two or more point mutations in the kinase domain of BCR-ABL are detectable by direct sequencing. In the case of polyclonal mutations, these BCR-ABL mutations (○ and △; top panel) exist separately in different clones. In contrast, BCR-ABL compound mutants exhibit two mutations within the same *BCR-ABL1* molecule (○ and △; bottom panel).

associated with the presence of multiple mutations. Soverini et al suggested that the high degree of complexity uncovered by UDS indicates that conventional DS might be an inadequate tool to assess BCR-ABL kinase domain mutation status, which currently represents an important component of the therapeutic decision algorithms.

Development of novel agents to override imatinib-resistant mutations

In order to override these imatinib resistance mechanisms, high-dose imatinib therapy [42] or combination treatment with other agents [43-45] has been performed. Imatinib has no therapeutic efficacy against patients harboring ABL point mutations; therefore, a novel agent that can also bind to mutated ABL has been vigorously developed.

Second generation ABL TKIs and mutations

Dasatinib: Affinity of dasatinib for ABL is about 325-fold stronger than that of imatinib. It inhibits more than 50 kinases including eight SRC Family Kinases (SFKs) as well as BCR-ABL, resulting that its specificity is relatively low [46]. Dasatinib is effective for most mutant BCR-ABLs including Y253F/H and F359C/V, which cannot be eliminated by nilotinib, its effects on T315I/A, F317L/V, V299L, Q252H are limited [10,15].

Nilotinib: With the structural modification of imatinib, nilotinib acquired about a 30-fold stronger anti-CML activity. It is effective against many mutant BCR-ABLs except T315I. Nilotinib suppresses ABL, PDGFR, and c-KIT activities but does not suppress SFKs, indicating higher specificity than dasatinib [11].

Dasatinib and nilotinib were approved by Food and Drug Administration (FDA) for second-line treatment of CML in 2006 and 2007, respectively, and both were approved for first-line treatment in 2010 based on efficacy and safety in phase III clinical studies. Based on the preclinical (Table 1, Table 2) [14-15] and clinical trials, imatinib-resistant/intolerant patients are treated with either dasatinib or nilotinib selectively according to the mutation type and patients' backgrounds (Figure 6) [47]. The incidence of resistance to front-line TKI treatment is generally lower with nilotinib and dasatinib than with imatinib, although up to 20% of patients may still be affected [48,49].

Bosutinib: Bosutinib has about a 50-fold stronger activity against ABL compared with imatinib and a characteristically weak inhibitory activity against c-KIT or PDGFR, which is a cause of side effects in imatinib. Although bosutinib is also effective against most mutant BCR-ABLs, it is not effective against T315I and V299Lsame as dasatinib [12]. Bosutinib was approved by the FDA in September 2012. In a Phase I/II, nonrandomized clinical trial, similar responses were observed across all BCR-ABL KD mutations when compared with wild-type BCR-ABL, with the exception of the highly resistant T315I mutation. Patients with the bosutinib-resistant V299L mutation were not included in the study [50].

Bafetinib (formerly INNO-406): Bafetinib is 55 times more effective than imatinib. Bafetinib can suppress 12 out of 13 mutated forms of BCR-ABL, with the exception of T315I. Furthermore, bafetinib inhibits LYN activity at the enzyme level without affecting the phosphorylation of SRC, BLK and YES [13]. In an *in vivo* investigation, bafetinib prolonged the survival phase of leukemic model mice inoculated with Ba/F3 cell lines derived from murine pro-B cells expressing either wild type

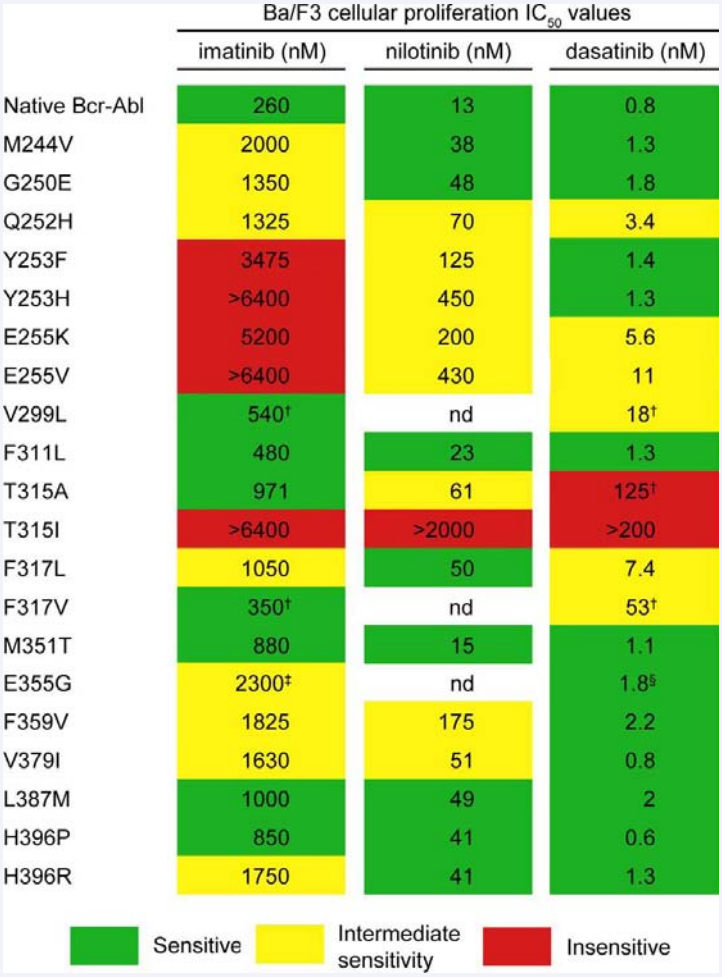

Table 1 Sensitivity of Bcr-Abl kinase domain mutants to Abl kinase inhibitors.

Imatinib: sensitive (≤1000 nM), intermediate (≤3000 nM), insensitive (>3000 nM). Nilotinib: sensitive (≤50 nM), intermediate (≤500 nM), insensitive (>500 nM). Dasatinib: sensitive (≤3 nM), intermediate (≤60 nM), insensitive (>60 nM) [14].

Table 2: Imatinib, dasatinib, nilotinib and bafetinib IC_{50} values (nM) for cellular proliferation [15].

	imatinib	dasatinib	nilotinib	bafetinib
Ba/F3/wt	1712.0	87.6	642.3	152.9
Ba/F3/M244V	>2,000	93.1	891.4	874.2
Ba/F3/G250E	>2,000	27.2	866.0	852.0
Ba/F3/Q252H	>2,000	62.3	895.5	888.1
Ba/F3/Y253F	>2,000	16.8	975.3	490.8
Ba/F3/T315I	>2,000	>2,000	>2,000	>2,000
Ba/F3/T315A	>2,000	>2,000	949.2	422.5
Ba/F3/F317L	>2,000	>2,000	929.8	293.5
Ba/F3/F317V	1053.7	>2,000	286.9	284.0
Ba/F3/M351T	>2,000	88.0	580.4	582.9
Ba/F3/H396P	>2,000	8.9	986.9	280.1

BCR-ABL or mutated BCR-ABL including M244V, G250E, Q252H, Y253F, E255K, M351T and H396P, except T315I [51]. Bafetinib can inhibit T315A and F317L/V, while dasatinib was ineffective against the mutated ABL at amino acids 315 and 317 [15]. In a phase I study, bafetinibhad anti-CML efficacy in a heavily pretreated study population [52]. Interestingly, we recently reported the possibility that bafetinib is effective not only CML but also Parkinson's disease [53].

TYPE AND NUMBER OF BCR-ABL KINASE DOMAIN

Mutation status does not always predict the response of patients to second-line treatment except for a small number of mutations for which clinical experience has confirmed a correlation between in vitro data and response in patients [54]. As shown in Table 3 some patients harboring mutations that are considered "nilotinib-insensitive" (eg, E255K) or "dasatinib-insensitive" (eg, F317L) and "multi-TKI–insensitive" (ie, T315I) can show clinical response to nilotinib, dasatinib, and more than one TKI, respectively, albeit at a rate lower than that seen with the agents not associated with resistance to these mutations.

Agents for T315I

It is irony for T315I which is firstly discovered [6] to remain troublesome even with any second generation ABL TKIs. Thus, the T315I mutation is considered a clinically significant mutation because until recently, patients harboring this mutation had very few treatment options. Very recently, some novel agents can be used now in clinic.

Ponatinib (Iclusig™): Ponatinib is a third-generation BCR-ABL TKI. It is effective against most mutant BCR-ABLs including T315I. It can avoid the conformational effect of T315I because of a long and flexible carbon-carbon triple bond (Figure 7) [55,56]. Ponatinib is presently the only clinically available ABL TKI effective against T315I. However, caution is needed as it may be ineffective against compound mutations [27]. The FDA approved ponatinib in December 2012.

In the PACE study, with a median of 12 months' follow-up, 45 (70%) of 64 patients with CML-CP and the T315I mutation achieved MCyR on ponatinib [57]. In a subanalysis of the PACE trial, MCyR was achieved regardless of baseline mutation status. Responses were also observed in patients with mutations other than T315I (eg, E255V, F359V, Y253H).

PF-114: PF-114 is an orally available novel selective pan BCR-ABL inhibitor targets the T315I and suppresses models of advanced Ph^+ALL. PF-114 is more selective than dasatinib or ponatinib. It efficiently inhibited all tested BCR-ABL mutants in cellular and biochemical assays at dosages of 10-100nM and like ponatinib it suppressed the up-coming of new resistance mutations in a mutation assay in Ba/F3 cells [58]. At present (March 2014), clinical trials of PF-114 have not started yet.

Omacetaxicine (Synribo™**):** Homoharringtonine (HHT), a cephalotaxine ester inhibits protein synthesis and induces cell differentiation and apoptosis. The absence of cross-resistance between HHT and imatinib in imatinib-resistant leukemic cell lines has been demonstrated, and even a possible synergy between the two compounds has been observed *in vitro*. Clinical outcomes for CML patients who acquired the T315I mutation while on imatinib were reported. Omacetaxine is a treatment option for patients with the T315I mutation for whom ponatinib

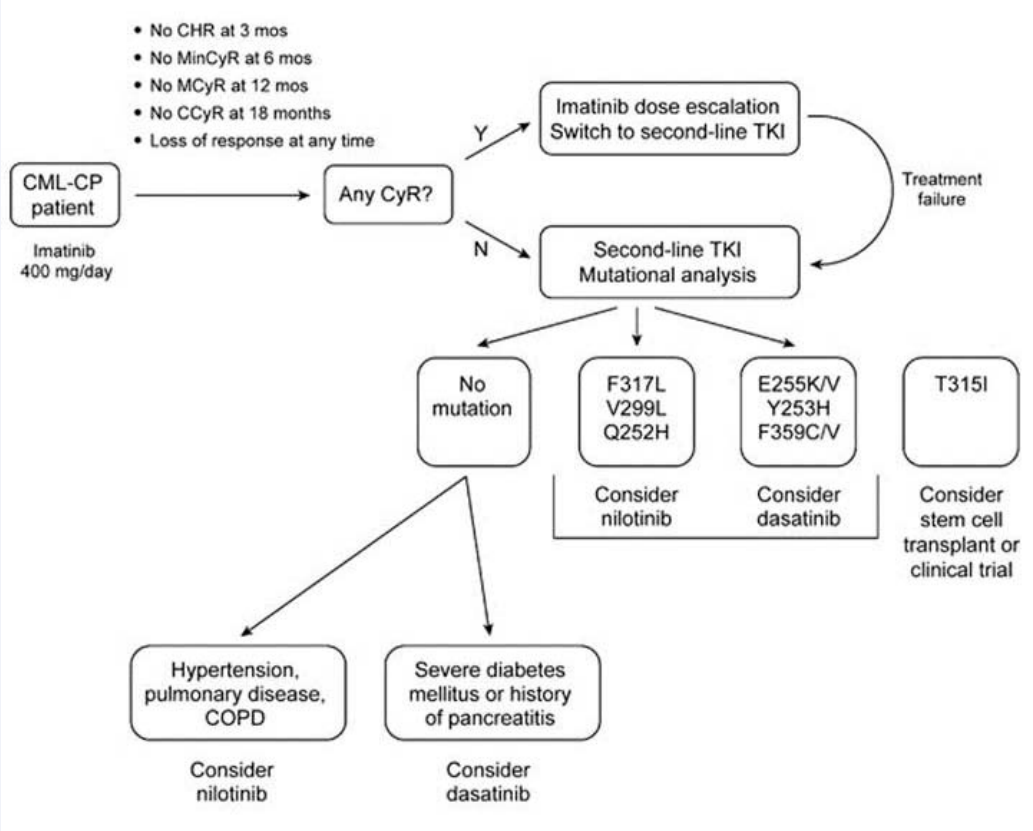

Figure 6 Treatment approach for patients with chronic myeloid leukemia in chronic phase (CML-CP).

CyR, cytogenetic response; CCyR, complete cytogenetic response; CHR, complete hematological response; COPD, chronic obstructive pulmonary disease; MCyR, major cytogenetic response; minCyR, minor cytogenetic response; TKI, tyrosine kinase inhibitor [47].

	Dasatinib[87]			Nilotinib[87]				Bosutinib[51]			
	(n = 92)			(n = 120)		(n = 192)		(n = 61)		(n = 94)	
Mutation	n[b]	%CHR	%MCyR	n	%CHR	n	%MCyR	n	%CHR	n	%MCyR
No Mutation	51	98	55	58	82	105	51	27	100	35	54
Any Mutation[c]	41	88	46	62	61	77	42	34	97	59	69
M244V	9	89	56	8	87	10	70	2	100	3	67
L248V	2	100	50	1	0	2	0	3	100	3	67
G250E	7	86	57	4	75	4	75	1	100	2	100
Y253F	–	–	–	1	100	1	0	1	100	1	0
Y253H	1	0	0	5	0	7	14	1	100	1	100
E255K	–	–	–	5	40	5	20	2	100	2	50
E255V	–	–	–	1	0	1	0	1	100	1	100
E274K	–	–	–	1	0	1	0	–	–	–	–
E275K	–	–	–	1	100	1	0	–	–	–	–
D276G	–	–	–	1	100	2	50	1	100	1	0
E279K	–	–	–	2	100	2	50	–	–	–	–
L298V	–	–	–	–	–	–	–	1	100	1	0
F311I	–	–	–	–	–	–	–	1	100	1	100
F311L	–	–	–	1	100	1	0	1	100	1	100
T315I	3	33	33	4	0	4	0	2	50	3	0
F317L	4	100	25	2	50	2	0	0	NA	3	100
N331S	–	–	–	–	–	–	–	0	NA	1	100
M351T	6	100	33	9	66	11	54	3	100	6	100
E355A	–	–	–	0	NA	1	100	–	–	–	–
E355G	–	–	–	1	100	3	67	2	100	2	50
F359I	–	–	–	2	50	2	0	1	100	1	100
F359V	4	100	25	7	43	8	38	6	100	7	57
L364P	–	–	–	–	–	–	–	0	NA	1	100
L370P	–	–	–	0	NA	1	100	–	–	–	–
L387F	–	–	–	–	–	–	–	0	NA	1	0
L387M	–	–	–	1	100	1	0	–	–	–	–
M388I	–	–	–	1	100	1	100	–	–	–	–
H396P	–	–	–	–	–	–	–	1	100	2	100
H396R	3	67	33	4	75	5	60	1	100	0	NA
I418V	1	100	100	–	–	–	–	–	–	–	–
D421G	–	–	–	–	–	–	–	1	100	1	0
I432T	–	–	–	–	–	–	–	0	NA	1	0
S438C	–	–	–	2	100	2	0	–	–	–	–
E450K	–	–	–	1	–[d]	1	100	–	–	–	–
E450V	–	–	–	–	–	–	–	1	100	1	100
E453K	–	–	–	–	–	–	–	0	NA	1	100
E453Q	–	–	–	–	–	–	–	1	100	1	100
E459G	–	–	–	1	100	1	100	–	–	–	–
E459K	–	–	–	1	100	2	0	0	NA	1	0
P480A	–	–	–	–	–	–	–	0	NA	1	100
F486S	–	–	–	2	50	2	0	–	–	–	–

Abbreviations: – = mutation not detected, not analyzed, or not reported; CHR = complete hematologic response; MCyR = major cytogenetic response; NA = not applicable.
[a]Response by mutation has not been reported for second-line ponatinib therapy.
[b]Data were reported only for mutations that were detected in 2 or more patients in the study.
[c]Some patients may have had more than one mutation.
[d]Response data for one patient with E450K were not reported.

Table 3 Summary of response to second-line TKI therapy in patients with resistance to first-line imatinib, by ABL kinase domain mutation [54].
Abbreviations: -: mutations not dected, not analyzed, or not reported; CHE: Complete Hematologic Response; MCYR: Major Cytogenetic Respose; NA= Not applicable.
[a]Response by mutations has not been reported for second-line ponatinib therapy.
[b]Data were reported only for mutations that were detected in 2 or more patients in the study.
[c]some patients may have had more than one mutation.
[d]Response data for one patient with E450K were not reported.

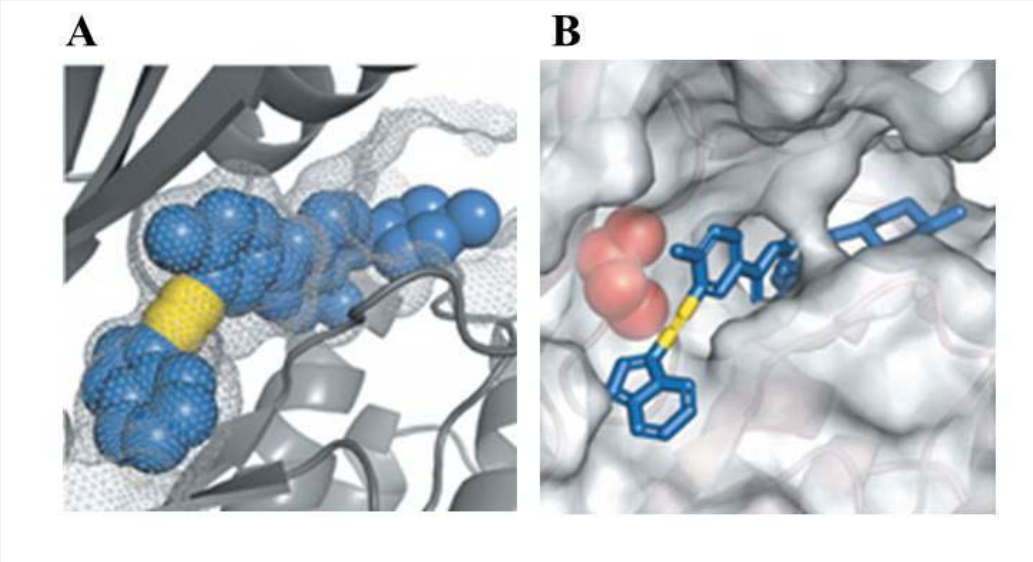

Figure 7 Structure of ponatinib.

(A) Ponatinib (shown as blue and yellow space-filling spheres) displays an optimal fit to the binding cavity of AVL-T315I (indicated by a mesh pattern), (B) Triple bond (yellow) is a unique structural feature of ponatinib (blue) that allows it to evade the mutant gatekeeper residue I315 (red space-filling spheres) [56].

is not suitable [54]. In a study involving 62 patients with CML-CP who were harboring the T315I mutation after ≥ 1 TKI that included imatinib, treatment with omacetaxine resulted in CHR in 77%, MCyR in 23%, and CCyR in 16% of patients [59].

Other novel agents: The above agents other than ponatinib and omacetaxine mepesuccinate are ineffective against CML cells and CML stem cells with T315I, and efforts to develop novel agents are being made to overcome this weakness. Aurora kinase is a serine/threonine kinase closely involved in cell division. Danusertib (PHA-739358) [60], AT9283 [61], and XL228are its variations with inhibitory activities against wild and various mutant BCR-ABLs. Also, drugs called switch pocket inhibitors are being developed and undergoing clinical trials. As drugs that directly act on CML stem cells, there are hedgehog inhibitors [62], histone deacetylase inhibitors [63], heat shock protein 90 inhibitors [64], and vaccines such as WT1 peptide [65] and K562/GM-CSF vaccines [66].

CONCLUSION

Although mutation at ABL KD is not only mechanism of ABL TKIs resistance, it could be most important. To detect it and treat CML patients according to the mutation is essential for using ABL TKIs and other novel agents properly.

CONFLICT OF INTEREST

Dr. Kimura reports receiving grant support from Bristol-Myers Squibb, Novartis and Pfizer.

REFERENCES

1. Sawyers CL. Chronic myeloid leukemia. N Engl J Med. 1999; 340: 1330-1340.
2. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003; 348: 994-1004.
3. Druker BJ. Perspectives on the development of imatinib and the future of cancer research. Nat Med. 2009; 15: 1149-1152.
4. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med. 2001; 344: 1038-1042.
5. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, et al. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood. 2002; 100: 1965-1971.
6. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001; 293: 876-880.
7. Hofmann WK, Jones LC, Lemp NA, de Vos S, Gschaidmeier H, Hoelzer D, et al. Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. Blood. 2002; 99: 1860-1862.
8. Nardi V, Azam M, Daley GQ. Mechanisms and implications of imatinib resistance mutations in BCR-ABL. Curr Opin Hematol. 2004; 11: 35-43.
9. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 2005; 105: 2640-2653.
10. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004; 305: 399-401.
11. Weisberg E, Manley PW, Breitenstein W, Brüggen J, Cowan-Jacob SW, Ray A, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell. 2005; 7: 129-141.
12. Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, et al.

SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. Cancer Res. 2003; 63: 375-381.

13. Kimura S, Naito H, Segawa H, Kuroda J, Yuasa T, Sato K, et al. NS-187, a potent and selective dual Bcr-Abl/Lyn tyrosine kinase inhibitor, is a novel agent for imatinib-resistant leukemia. Blood. 2005; 106: 3948-3954.

14. O'Hare T, Eide CA, Deininger MW. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. Blood. 2007; 110: 2242-2249.

15. Deguchi Y, Kimura S, Ashihara E, Niwa T, Hodohara K, Fujiyama Y, et al. Comparison of imatinib, dasatinib, nilotinib and INNO-406 in imatinib-resistant cell lines. Leuk Res. 2008; 32: 980-983.

16. Redaelli S, Piazza R, Rostagno R, Magistroni V, Perini P, Marega M, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. J Clin Oncol. 2009; 27: 469-471.

17. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell. 2009; 16: 401-412.

18. Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, et al. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up: Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib. Blood (ASH Annual Meeting Abstracts). 2009; 114: 1126.

19. Hegedus T, Orfi L, Seprodi A, Váradi A, Sarkadi B, Kéri G. Interaction of tyrosine kinase inhibitors with the human multidrug transporter proteins, MDR1 and MRP1. Biochim Biophys Acta. 2002; 1587: 318-325.

20. Wu J, Meng F, Lu H, Kong L, Bornmann W, Peng Z, et al. Lyn regulates BCR-ABL and Gab2 tyrosine phosphorylation and c-Cbl protein stability in imatinib-resistant chronic myelogenous leukemia cells. Blood. 2008; 111: 3821-3829.

21. White DL, Dang P, Engler J, Frede A, Zrim S, Osborn M, et al. Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. J Clin Oncol. 2010; 28: 2761-2767.

22. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci U S A. 2006; 103: 14907-14912.

23. Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med. 2012; 18: 521-528.

24. Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F. A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PLoS One. 2013; 8: e78582.

25. Shah NP, Nicoll JM, Nagar B,Gorre ME, Paquette RL, Kuriyan J, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell. 2002; 2: 117-125.

26. Roumiantsev S, Shah NP, Gorre ME, Nicoll J, Brasher BB, Sawyers CL, et al. Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. Proc Natl Acad Sci U S A. 2002; 99: 10700-10705.

27. Khorashad JS, Kelley TW, Szankasi P, Mason CC, Soverini S, Adrian LT, et al. BCR-ABL1 compound mutations in tyrosine kinase inhibitor-resistant CML: frequency and clonal relationships. Blood. 2013; 121: 489-498.

28. Sherbenou DW, Hantschel O, Kaupe I, Willis S, Bumm T, Turaga LP, et al. BCR-ABL SH3-SH2 domain mutations in chronic myeloid leukemia patients on imatinib. Blood. 2010; 116: 3278-3285.

29. Lee TS, Ma W, Zhang X, Giles F, Cortes J, Kantarjian H, Albitar M. BCR-ABL alternative splicing as a common mechanism for imatinib resistance: evidence from molecular dynamics simulations. Mol Cancer Ther. 2008; 7: 3834-3841.

30. O'Hare T, Zabriskie MS, Eide CA, Agarwal A, Adrian LT, You H, Corbin AS. The BCR-ABL35INS insertion/truncation mutant is kinase-inactive and does not contribute to tyrosine kinase inhibitor resistance in chronic myeloid leukemia. Blood. 2011; 118: 5250-5254.

31. Carella AM, Garuti A, Cirmena G, Catania G, Rocco I,Palermo C, et al. Kinase domain mutations of BCR-ABL identified at diagnosis before imatinib-based therapy are associated with progression in patients with high Sokal risk chronic phase chronic myeloid leukemia. Leuk Lymphoma. 2010; 5: 275–278.

32. Chu S, Xu H, Shah NP, Snyder DS, Forman SJ, Sawyers CL, Bhatia R. Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment. Blood. 2005; 105: 2093-2098.

33. Iqbal Z, Aleem A, Iqbal M, Naqvi MI, Gill A, Taj AS, et al. Sensitive Detection of Pre-Existing BCR-ABL Kinase Domain Mutations in CD34+ Cells of Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia Patients Is Associated with Imatinib Resistance: Implications in the Post-Imatinib Era. PLoSOne. 2013; 8: e55717.

34. Vannucchi AM, Pancrazzi A, Bogani C, Antonioli E, Guglielmelli P. A quantitative assay for JAK2(V617F) mutation in myeloproliferative disorders by ARMS-PCR and capillary electrophoresis. Leukemia. 2006; 20: 1055-1060.

35. Soverini S, Martinelli G, Amabile M, Poerio A, Bianchini M, Rosti G, et al. Denaturing-HPLC-based assay for detection of ABL mutations in chronic myeloid leukemia patients resistant to Imatinib. Clin Chem. 2004; 50: 1205-1213.

36. Gruber FX, Lamark T, Anonli A, Sovershaev MA, Olsen M, Gedde-Dahl T, et al. Selecting and deselecting imatinib-resistant clones: observations made by longitudinal, quantitative monitoring of mutated BCR-ABL. Leukemia. 2005; 19: 2159-2165.

37. Khorashad JS, Anand M, Marin D, Saunders S, Al-Jabary T, Iqbal A, et al. The presence of a BCR-ABL mutant allele in CML does not always explain clinical resistance to imatinib. Leukemia. 2006; 20: 658-663.

38. Kreuzer KA, Le Coutre P, Landt O, Na IK, Schwarz M, Schultheis K, et al. Preexistence and evolution of imatinib mesylate-resistant clones in chronic myelogenous leukemia detected by a PNA-based PCR clamping technique. Ann Hematol. 2003; 82: 284-289.

39. Martinez-Serra J, Gutiérrez A, Marcús TF, Soverini S, Amat JC, Navarro-Palou M, et al. Four-channel asymmetric Real-Time PCR hybridization probe assay: a rapid pre-screening method for critical BCR-ABL kinase domain mutations. Clin Biochem. 2012; 45: 345-351.

40. Tanaka R, Kimura S, Ashihara E, Yoshimura M, Takahashi N, Wakita H, et al. Rapid automated detection of ABL kinase domain mutations in imatinib-resistant patients. Cancer Lett. 2011; 312: 228-234.

41. Soverini S, De Benedittis C, Machova Polakova K, Brouckova A, Horner D, Iacono M, et al. Unraveling the complexity of tyrosine kinase inhibitor-resistant populations by ultra-deep sequencing of the BCR-ABL kinase domain. Blood. 2013; 122: 1634-1648.

42. Kantarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, Giles F, et al. High-dose imatinib mesylate therapy in newly diagnosed Philadelphia chromosome-positive chronic phase chronic myeloid leukemia. Blood. 2004; 103: 2873-2878.

43. Kuroda J, Kimura S, Segawa H, Kobayashi Y, Yoshikawa T, Urasaki Y, et al. The third-generation bisphosphonate zoledronate synergistically augments the anti-Ph+ leukemia activity of imatinib mesylate. Blood. 2003; 102: 2229-2235.

44. Angstreich GR, Matsui W, Huff CA, Vala MS, Barber J, Hawkins AL, et al. Effects of imatinib and interferon on primitive chronic myeloid leukaemia progenitors. Br J Haematol. 2005; 130: 373-381.

45. Oki Y, Kantarjian HM, Gharibyan V, Jones D, O'brien S, Verstovsek S, et al. Phase II study of low-dose decitabine in combination with imatinib mesylate in patients with accelerated or myeloid blastic phase of chronic myelogenous leukemia. Cancer. 2007; 109: 899-906.

46. Hantschel O, Rix U, Superti-Furga G. Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. Leuk Lymphoma. 2008; 49: 615-619.

47. Jabbour E, Hochhaus A, Cortes J, La Rosée P, Kantarjian HM. Choosing the best treatment strategy for chronic myeloid leukemia patients resistant to imatinib: weighing the efficacy and safety of individual drugs with BCR-ABL mutations and patient history. Leukemia. 2010; 24: 6-12.

48. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010; 362: 2251-2259.

49. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010; 362: 2260-2270.

50. Cortes JE, Kantarjian HM, Brümmendorf TH, Kim DW, Turkina AG, Shen ZX, et al. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome-positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood. 2011; 118: 4567-4576.

51. Naito H, Kimura S, Nakaya Y, Naruoka H, Kimura S, Ito S, Wakayama T. In vivo antiproliferative effect of NS-187, a dual Bcr-Abl/Lyn tyrosine kinase inhibitor, on leukemic cells harbouring Abl kinase domain mutations. Leuk Res. 2006; 30: 1443-1446.

52. Kantarjian H, le Coutre P, Cortes J, Pinilla-Ibarz J, Nagler A, Hochhaus A, et al. Phase 1 study of INNO-406, a dual Abl/Lyn kinase inhibitor, in Philadelphia chromosome-positive leukemias after imatinib resistance or intolerance. Cancer. 2010; 116: 2665-2672.

53. Imam SZ, Trickler W, Kimura S, Binienda ZK, Paule MG, Slikker W Jr, et al. Neuroprotective efficacy of a new brain-penetrating C-Abl inhibitor in a murine Parkinson's disease model. PLoS One. 2013; 8: e65129.

54. Lau A, Seiter K. Second-line therapy for patients with chronic myeloid leukemia resistant to first-line imatinib. Clin Lymphoma Myeloma Leuk. 2014; 14: 186-196.

55. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell. 2009; 16: 401-412.

56. Cortes JE, Kantarjian H, Shah NP, Bixby D, Mauro MJ, Flinn I, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. N Engl J Med. 2012; 367: 2075-2088.

57. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013; 369: 1783-1796.

58. Mian A, Rafiei A, Metodieva A, Haberbosch I, Zeifman A, Titov I, et al. PF-114, a Novel Selective Pan BCR/ABL Inhibitor Targets The T315I and Suppress Models Of Advanced Ph+ ALL. Blood (ASH Annual Meeting Abstracts). 2013; 122: 3907.

59. Cortes J, Lipton JH, Rea D, Digumarti R, Chuah C, Nanda N, et al. Phase 2 study of subcutaneous omacetaxine mepesuccinate after TKI failure in patients with chronic-phase CML with T315I mutation. Blood. 2012; 120: 2573-2580.

60. Gontarewicz A, Balabanov S, Keller G, Colombo R, Graziano A, Pesenti E, et al. Simultaneous targeting of Aurora kinases and Bcr-Abl kinase by the small molecule inhibitor PHA-739358 is effective against imatinib-resistant BCR-ABL mutations including T315I. Blood. 2008; 111: 4355-4364.

61. Tanaka R, Squires MS, Kimura S, Yokota A, Nagao R, Yamauchi T, et al. Activity of the multitargeted kinase inhibitor, AT9283, in imatinib-resistant BCR-ABL-positive leukemic cells. Blood. 2010; 116: 2089-2095.

62. Katagiri S, Tauchi T, Okabe S, Minami Y, Kimura S, Maekawa T, et al. Combination of ponatinib with Hedgehog antagonist vismodegib for therapy-resistant BCR-ABL1-positive leukemia. Clin Cancer Res. 2013; 19: 1422-1432.

63. Okabe S, Tauchi T, Kimura S, Maekawa T, Kitahara T, Tanaka Y, et al. Combining the ABL1 kinase inhibitor ponatinib and the histone deacetylase inhibitor vorinostat: a potential treatment for BCR-ABL-positive leukemia. PLoS One. 2014; 9: e89080.

64. Tauchi T, Okabe S, Ashihara E, Kimura S, Maekawa T, Ohyashiki K. Combined effects of novel heat shock protein 90 inhibitor NVP-AUY922 and nilotinib in a random mutagenesis screen. Oncogene. 2011; 30: 2789-2797.

65. Oji Y, Oka Y, Nishida S, Tsuboi A, Kawakami M, Shirakata T, et al. WT1 peptide vaccine induces reduction in minimal residual disease in an Imatinib-treated CML patient. Eur J Haematol. 2010; 85: 358-360.

66. Smith BD, Kasamon YL, Kowalski J, Gocke C, Murphy K, Miller CB, et al. K562/GM-CSF immunotherapy reduces tumor burden in chronic myeloid leukemia patients with residual disease on imatinib mesylate. Clin Cancer Res. 2010; 16: 338-347.

Cite this article

Kimura S, Ando T, Kojima K (2014) BCR-ABL Point Mutations and TKI Treatment in CML Patients. J Hematol Transfus 2(3): 1022.