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MEDICAL PRACTICE

BCR-ABL mutational studies for predicting the response of patients with chronic myeloid leukaemia to second-generation tyrosine kinase inhibitors after imatinib failure

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Imatinib is the standard treatment for chronic myeloid leukaemia. BCR-ABL kinase domain mutation is the commonest mechanism implicated in imatinib resistance. In in-vitro studies, kinase domain mutations are variably resistant to second-line agents. We performed BCR-ABL kinase domain mutational studies in 25 patients in five institutions who failed imatinib and were treated with either nilotinib or dasatinib, to see if their mutational status would predict their clinical responses. Kinase domain mutations involving 11 amino acid substitutions were found in 12 (48%) patients. Most patients showed single kinase domain mutations. There was some concordance between reported drug sensitivity patterns and patient responses. Discordant responses could be related to drug dosage variations and unknown BCR-ABL independent mechanisms. The response prediction for patients with multiple kinase domain mutations was challenging and their mutational patterns could change after tyrosine kinase inhibitor therapy. Although BCR-ABL kinase domain mutational analysis has limitations as a means of predicting the clinical response to second-line tyrosine kinase inhibitors, it helps inform therapy decisions in the management of chronic myeloid leukaemia after imatinib failure.

Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferative neoplasm of transformed pluripotent haematopoietic progenitors. The Philadelphia (Ph) chromosome that results from the balanced translocation t(9;22)(q11;q34) fusing with the ABL proto-oncogene on chromosome 9 and the *BCR* gene on chromosome 22 is the cytogenetic hallmark of the disease. Chronic myeloid leukaemia is a three-phase disease. Most patients present in the chronic phase (CP), and progress, years later, to the blast phase (BP), either directly or through an intermediate accelerated phase (AP). Bone marrow transplantation (BMT) is the only curative option but this is applicable only to young patients with available histocompatible donors and carries significant morbidity and mortality. Hydroxyurea and busulfan control myeloproliferation but not natural progression of the disease. Although interferon-α (IFN-α) prolongs survival, the cytogenetic response rate is low and there are problems with poor drug tolerance.

Imatinib mesylate (Glivec or Gliveec, Novartis Pharmaceuticals, Basel, Switzerland) is the tyrosine kinase inhibitor (TKI) that inhibits the BCR-ABL oncoprotein. It is more effective than IFN-α, and safer than allogenic stem cell transplantation for controlling CML.[1] After 5 years, the best rates of complete haematologic response (CHR), complete cytogenetic response (CCgR), and overall survival were 97%, 87% and 89% respectively, which are higher than any of the conventional therapies for CML.[2,3] It has been approved by the Hong Kong Hospital Authority as a first-line agent for the treatment of CML since 2005.

Although the majority of CML patients show haematological responses, approximately 3% of CP, 20% of AP, and 50% of BP patients never respond.[2,4,5] Moreover, responders relapse at a rate of 0.4 to 5.5% annually.[2] Mechanisms contributing to imatinib resistance are heterogeneous, which can be BCR-ABL–dependent, such as ABL kinase domain (KD) point mutation,[6] BCR-ABL gene amplification,[6] and BCR-ABL–independent mechanisms such as activation of alternative kinases[7] and changes in drug influx (*hOCT1*) or efflux (*MDR1*) proteins.[8] Of these mechanisms, BCR-ABL KD point mutation is the most common and the best understood.[9]

Imatinib binds specifically to the ATP-binding pocket on the catalytic domain of the inactive-conformation BCR-ABL oncoprotein.[10] Point mutations that change the amino acid of the contact site or the specific KD conformation to which imatinib binds

Key words
Leukemia, myelogenous, chronic, BCR-ABL positive; Mutation; Protein kinase inhibitors; protein-tyrosine kinases

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以BCR-ABL突變研究，預測慢性骨髓白血病患者無法以伊馬替尼治療後，對使用第二線酚基乙氨酸激酶抗化劑的反應

伊馬替尼（Imatinib）為慢性骨髓白血病的治療標準。當患者對伊馬替尼有抗藥性，大多顯示出現BCR-ABL激酶區突變。根據體外研究，激酶區突變多對二線藥物有抗藥性。本文分析於五所醫院共25名對伊馬替尼有抗藥性，改以尼羅替尼（nilotinib）或達沙替尼（dasatinib）治療的患者進行的BCR-ABL激酶區突變研究，檢視他們的突變狀態是否有助預測他們的臨床反應。結果顯示，12名（48%）患者出現牽涉激酶11氨基酸的激酶區突變；多數患者顯示單體激酶區突變。藥物敏感模式和患者反應是一致的；但不協調反應跟藥物劑量變異與未知的BCR-ABL獨立機制有關。預測有多個激酶區突變患者的臨牀反應並不容易，而且他們的突變模式或因酚基乙氨酸激酶抗化劑療法後改變。雖然BCR-ABL激酶區突變分析對預測二線酚基乙氨酸激酶抗化劑的臨床反應作用有限，但仍有助決定在伊馬替尼失效後慢性骨髓白血病的治療方案。

attenuate its inhibitory property.[11,12] More than 90 mutations have been reported, most of them in four regions: the ATP-binding loop (P-loop), the contact site, the *SH2*-binding site (activation loop), and the catalytic domain.[13] Most mutations involve amino acid substitutions at a few residues (P-loop: E255/V, M244V, G250E, Y253H/F; drug contact site: T315I, F317L; catalytic domain: M351T, F359V).[14] They vary in the degree of drug resistance and clinical significance.[15,16] Some mutants may still be sensitive to a higher imatinib concentration,[17,18] while others that are highly resistant are only inhibited by more potent second-generation TKIs.[19]

Nilotinib (Tasigna, Novartis Pharmaceuticals, Basel, Switzerland) and dasatinib (Sprycel, Bristol-Myers Squibb, Princeton [NJ], US) are the second-generation TKIs approved by the Food and Drug Administration for treatment of imatinib-resistant and -intolerant CP and AP CML. Only dasatinib is approved for treatment of blastic disease. Nilotinib is 30 times, and dasatinib is 300 times more potent than imatinib.[19] Dasatinib differs from imatinib and nilotinib in that it binds to both active and inactive conformations of ABL.[19] It is also a dual kinase inhibitor active against both ABL and *Src* family kinases such as *Lyn*.[19] In in-vitro studies, most KD mutants show different sensitivities to nilotinib and dasatinib except for T315I, which is highly resistant to both agents.[20] Mutations resistant to one TKI agent may still be responsive to others.[21,22] Therefore, when CML fails to respond to TKI therapy, a mutational analysis may help guide the decision to switch to alternative TKI agents or another therapy. In a cohort of CML patients failing imatinib and other TKIs, we performed a retrospective analysis for BCR-ABL KD mutation before further treatment with alternative agents. The relationship between the response and the KD mutations was studied.

Methods

Samples were collected from CML patients resistant to imatinib or other TKIs between October 2006 and September 2008. BCR-ABL KD mutational assays were conducted in the Queen Mary Hospital and the Hong Kong Sanatorium and Hospital. The group included patients referred from five regional tertiary centres in Hong Kong (Queen Mary Hospital, Hong Kong Sanatorium and Hospital, Tuen Mun Hospital, Princess Margaret Hospital, and Pamela Youde Nethersole Eastern Hospital).

Total RNA was extracted from the peripheral blood using a QIAamp RNA Blood Kit (Qiagen, Hilden, Germany). cDNA was then synthesised using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad [CA], US). The BCR-ABL KD was amplified by polymerase chain reaction (PCR), as previously described, using the same set of primers.[23] Polymerase chain reaction products were sequenced directly using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, US). The DNA sequences of the PCR products were then aligned against the reference sequence (GenBank accession number M14752) of the ABL KD using Mutation Surveyor software (Version 2.61, SoftGenetics, LLC, State College, US). Any presence of a mutation in a sample had to be confirmed in a second sample collected at the same time, if available, or in the same sample by repeated analysis.

Chronic phase, AP, and BP were defined according to the World Health Organization classification.[24] A CHR was defined as a white blood cell count of less than 10 000/mL, a platelet count of less than 450 000/mL, a basophil count of less than 5%, an absence of immature granulocytes in peripheral blood, and absence of splenic enlargement. A major cytogenetic response (MCgR) was defined as ≤35% Ph+ metaphase of 20 marrow metaphases examined using conventional cytogenetics, or ≤35% Ph+ nuclei of 300 interphase cells examined with fluorescence in-situ hybridisation using peripheral blood or marrow samples. A CCgR was defined as 0% Ph+ metaphase or interphase nuclei. A major molecular response (MMR) was defined as ≤0.1% BCR-ABL/ABL by real-time quantitative PCR (RQ-PCR) on the international scale (BCR-ABL^{IS}). A complete molecular response (CMR) was defined as an undetectable level of BCR-ABL transcript by RQ-PCR. With imatinib, primary haematologic resistance was defined as a failure to reach CHR after 3 months of treatment. Primary cytogenetic resistance was defined as a failure to reach MCgR after 18 months. Loss of MCgR, loss of CHR, and progression to AP or BP constituted secondary or acquired resistance.

Many patients cannot tolerate the standard

doses of dasatinib (100 mg/day) and nilotinib (800 mg/day). Haematological toxicity, fluid retention, and rash were among the commonest side-effects leading to dosage reduction and therapy interruption. The mean TKI doses tolerated by patients were considered when interpreting the responses of KD mutants. Tyrosine kinase inhibitor mean dose intensity was calculated by averaging the total dose taken throughout the therapy period until disease progression, loss of response or latest follow-up. Days when TKI was not given were calculated as a percentage of total therapy duration.

TABLE 1. Indications of latest salvage tyrosine kinase inhibitor (TKI) therapy for patients in the present study

Indications for second-line TKI therapy	No. of patients
Primary resistance	
Haematologic	4
Cytogenetic	13
Secondary resistance	
Progression to accelerated phase/blast phase	5
Loss of complete haematologic response	1
Loss of complete cytogenetic response	-
Intolerant to imatinib	2
Total	**25**

Results

Patient characteristics and kinase domain mutations

Twenty-five patients (13 CP, 7 AP of which 2 were in second CP at the time of salvage, and 5 BP of which 1 was in second CP at the time of salvage) were assayed for BCR-ABL KD mutation before their latest salvage TKI therapy (18 before dasatinib and 7 before nilotinib). Two had relapses after BMT. All patients were haematologically or cytogenetically resistant to imatinib, and two were also intolerant, preventing escalation to the standard dose (Table 1). Before dasatinib salvage, 11 had failed nilotinib treatment, and before nilotinib salvage, two had failed dasatinib or bosutinib.

Kinase domain point mutations resulting in substitution of 11 amino acid residues (M244V=1, L248V=1, G250E=1, Y253H=1, E255K=4, E279K=1, T315I=2, F317L=2, M351T=1, F359V=2, E459K=1) were found in 12 (48%) patients (Table 2). Most patients showed single KD mutations. Multiple KD mutations were found in four patients after sole imatinib therapy (2 CP and 2 BP).

Relationship between in-vitro drug sensitivities of kinase domain mutants and their clinical responses

In-vitro sensitivity to TKI of various KD mutants was assessed in terms of their IC_{50} values (Table 3). IC_{50} is the concentration of TKI required to inhibit 50% of Ba/F3 cell viability after transfection with BCR-ABL mutants.[27] We assessed whether the reported in-vitro drug sensitivity pattern of KD mutations can be used to predict clinical responses (Tables 2 and 3). IC_{50} values (Table 3) used in our study are adopted from previous publications by O'Hare et al (2005)[20] and Redaelli et al (2009).[27]

Two patients had F359V mutations (D1, D2). Both had failed previous imatinib and nilotinib therapy. After dasatinib, significant responses with MMR achieved at 18 months in D1 and CMR at 24 months in D2. The current starting dose recommended for dasatinib is 100 mg/day; 70 mg twice daily was the dose used in drug approval studies.[28,29] Patient D1 was only able to tolerate a lower dose of dasatinib (dose intensity, 55 mg/day). The high in-vitro sensitivity of F359V to dasatinib may allow dosage reduction for poorly tolerant patients without compromising clinical responses.

None of the patients with the highly resistant T315I mutant (D4, D17) responded to dasatinib (IC_{50} 75x). The patient with blastic disease (D17) had two other concomitant dasatinib-resistant mutants (E255K and F317L). Mutants E279K, M244V, and L248V were moderately resistant to nilotinib in vitro. Patients N5 and N6 with advanced disease harbouring these mutants were unresponsive to nilotinib, despite a nilotinib dose of 400 to 800 mg/day.

A single E255K mutation was observed in three patients (D10, D11, and D16). All had advanced disease and two had failed previous nilotinib therapy. After dasatinib, durable CHR and CCgR were achieved in patient D11, even though the E255K mutation is regarded as dasatinib-resistant, based on reported IC_{50} values. This patient had a higher dasatinib dose intensity (106 vs 31-52 mg/day) and fewer days off-medication (0% vs 56-61%) than the two non-responders who progressed to BP subsequently.

A single F317L mutation was seen in one patient (D3). This mutation is resistant to dasatinib in vitro and in vivo.[20,30,31] In a study of 12 patients with baseline F317L mutations, only three showed a transient haematological response to dasatinib.[31] Surprisingly, our patient with F317L responded dramatically and achieved MMR. The prescribed dose of dasatinib was not higher than that in published study (140 mg/day).[29] The reason for this patient's response is not clear, though one may speculate effect of dasatinib inhibition on kinases other than BCR-ABL such as *Lyn* which may be oncogenically more active and addictive in the leukaemia cells of some patients than in others.[7] In terms of in-vitro sensitivity, F317L should be more sensitive to nilotinib.[20]

TABLE 2. Summary of patients included in this study*

Patient No.	Prior TKI therapy	Disease phase at current TKI	Indication for current TKI	Mutation status before current TKI	Best haematologic response†
D1	Imatinib	CP	No MCgR	F359V	CHR
	Nilotinib				
D2	Imatinib	CP	No CHR	F359V	Achieved CHR
	Nilotinib				
D3	Imatinib	CP	Loss of CHR	F317L	Achieved CHR
D4	Imatinib	CP	No CHR	T315I	No CHR
N1	Imatinib	CP	No MCgR	Y253H	CHR
				E459K	
N2	Imatinib	CP	No CHR	G250E	CHR
				M351T	
D5	Imatinib	CP	No MCgR	No mutation	CHR
	Nilotinib				
D6	Imatinib	CP	No MCgR	No mutation	CHR
	Nilotinib				
D7	Imatinib	CP	No MCgR	No mutation	CHR
	Nilotinib				
D8	Imatinib	CP	No MCgR	No mutation	CHR
	Nilotinib				
D9	Imatinib	CP	No MCgR	No mutation	CHR
N3	Imatinib	CP	No MCgR	No mutation	CHR
	Bosutinib				
N4	Imatinib	CP	No MCgR	No mutation	CHR
	Dasatinib				
	Bosutinib				
D10	Imatinib	AP	No MCgR	E255K	CHR
	Nilotinib				
D11	Imatinib	AP	Progression to AP	E255K	Achieved CHR
N5	Imatinib	AP	Progression to AP	E279K	No CHR
D12	Imatinib	AP	Progression to AP	No mutation	Achieved CHR
D13	Imatinib	AP	Progression to AP	No mutation	Achieved CHR
D14	Imatinib	Post-allo-SCT AP in second CP	No MCgR	No mutation	CHR
	Nilotinib				
D15	Imatinib	Post-allo-SCT AP in second CP	No CHR	No mutation	Achieved CHR
	Nilotinib				
D16	Imatinib	BP	No MCgR	E255K	HR
	Nilotinib				
D17	Imatinib	BP	No CHR	E255K	No HR
				T315I	
				F317L	
N6	Imatinib	BP	Progression to BP	M244V	No HR
				L248V	
D18	Imatinib	BP	No HR	No mutation	No HR
	Nilotinib				
N7	Imatinib	BP in second CP	No MCgR	No mutation	CHR

* allo-SCT denotes allogenic stem cell transplantation, BP blast phase, CCgR complete cytogenetic response, CHR complete haematologic response, CP chronic phase, D patients on dasatinib therapy, HR haematological response, MCgR major cytogenetic response, MMR major molecular response, MUD-BMT marrow unrelated donor–bone marrow transplantation, N patients on nilotinib therapy, NE not evaluated, and TKI tyrosine kinase inhibitor

† Achieved CHR refers to CHR achieved in a patient without CHR at baseline

‡ Cytogenetic response was assessed using fluorescence in-situ hybridisation. For patients monitored by real-time quantitative polymerase chain reaction, level of cytogenetic response was estimated from BCR-ABL/ABL ratio[25,26]: No MCgR equivalence=BCR-ABL/ABL>12.7% (BCR-ABL^{IS}>10%); MCgR equivalence=BCR-ABL/ABL≤12.7% (BCR-ABL^{IS}≤10%); CCgR equivalence=BCR-ABL/ABL≤1.27% (BCR-ABL^{IS}≤1%); and MMR=BCR-ABL/ABL≤0.127% (BCR-ABL^{IS}≤0.1%)

§ Latest recommended starting dose for nilotinib 400 mg twice daily and dasatinib 100 mg once daily, respectively

Cytogenetic/molecular response‡	TKI mean dose intensity (mg/day)§	Days not on TKI (% of total duration)	Subsequent progression
CCgR equivalence	55	1%	No progression in CHR (22 months)
BCR-ABL/ABL: 44.38% (0 month) to MMR (18 months)			
CCgR	114	0%	No progression in CHR (27 months)
BCR-ABL/ABL: 69% (0 month) to CMR (24 months)			
CCgR	140	0%	No progression in CHR (27 months)
BCR-ABL/ABL: 65.3% (0 month) to MMR (24 months)			
No MCgR	103	6%	No progression in CHR (11 months)
BCR-ABL/ABL: 81.5% to 28.2% (10 months)			
MCgR	443	0%	No progression in CHR (25 months)
BCR-ABL/ABL: 28.7% (0 month) to 1.6% (25 months)			
No MCgR (8 months)	456	6%	Loss of CHR (8 months)
No MCgR equivalence	55	20%	MUD-BMT
BCR-ABL/ABL: 27.2% (0 month) to 35.7% (3 months)			
No MCgR	25	26%	No progression in CHR (22 months)
BCR-ABL/ABL: 69.8% (0 month) to 11.6% (22 months)			
NE	69	0%	Progression to BP (3 months)
No MCgR equivalence	53	10%	No progression in CHR (28 months)
BCR-ABL/ABL: 22% (0 month) to 15.1% (22 months)			
MCgR	35	5%	No progression in CHR (26 months)
BCR-ABL/ABL: 216% (0 month) to 11.6% (18 months)			
CCgR equivalence	534	7%	No progression in CHR (11 months)
BCR-ABL/ABL: 45.2% (0 month) to 0.46% (8 months)			
No MCgR equivalence	194	9%	No progression in CHR (9 months)
BCR-ABL/ABL: 24.96% (2 months) to 16.3% (7 months)			
No MCgR	52	61%	Progression to BP (7 months)
BCR-ABL/ABL: 94.2% (0 month) to 57.5% (6 months)			
CCgR	106	0%	No progression in CHR (24 months)
BCR-ABL/ABL: 197.8% to 0.2% (24 months)			
No CHR (3 months)	800	0%	No CHR (3 months)
MCgR equivalence	104	0%	Progression to BP (5 months)
BCR-ABL/ABL: 26% (0 month) to 9% (3 months)			
CCgR equivalence	44	11%	No progression in CHR (27 months)
BCR-ABL/ABL: 322% (0 month) to MMR (27 months)			
No MCgR	23	18%	No progression in CHR (24 months)
BCR-ABL/ABL: 229% (0 month) to 38.7% (24 months)			
No MCgR	49	9%	No progression in CHR (23 months)
BCR-ABL/ABL: 52.3% (0 month) to 10.9% (23 months)			
No MCgR	31	56%	Loss of HR with blastic relapse (7 months)
BCR-ABL/ABL: 64.2% (0 month) to 27.5% (6 months)			
NE	86	9%	Persistent BP (3 months)
NE	400	0%	Persistent BP (3 months)
NE	93	0%	Persistent BP (2 weeks)
NE	141	24%	Progression to BP (4 months)

TABLE 3. In-vitro sensitivity of kinase domain mutants to various tyrosine kinase inhibitors as measured by IC_{50} [20,27]

	Fold increase in IC_{50}*		
	Imatinib	Nilotinib	Dasatinib
Wild type	1	1	1
M244V	7.69	2.92	1.63
L248V	3.54	2.80	5.11
G250E	6.86	4.56	4.45
Y253H	24.62	34.62	1.63
E255K	6.02	6.69	5.61
E255V	16.99	10.31	3.44
E279K	3.55	2.05	1.64
T315I	17.50	39.41	75.03
F317L	2.60	2.22	4.46
M351T	1.76	0.44	0.88
F359V	2.86	5.16	1.49
E459K	NA	NA	NA

* Results are expressed as fold increase compared with that of wild-type BCR-ABL. IC_{50} values for imatinib, nilotinib, and dasatinib against wild-type BCR-ABL are 527 nM, 17.69 nM, and 1.83 nM respectively; relative resistance: ≤2 denotes sensitive, 2.01-4 moderately resistant, 4.01-10 resistant, and >10 highly resistant; NA denotes not available

Predicting responses in patients harbouring multiple KD mutations can be difficult, in particular those harbouring multiple mutants of discordant drug sensitivity. Again, the in-vitro drug sensitivity and the clinical response may not be concordant. Patient N2 showed concomitant nilotinib-sensitive M351T and -resistant G250E mutants. Major cytogenetic response was not reached and CHR was transient, lasting for only a period of 8 months. A repeated KD mutational analysis showed a shift in the pattern with the emergence of E255V and the disappearance of M351T and G250E. Patient N1 showed a concomitant nilotinib–highly resistant Y253H mutant, and an E459K mutant of unknown nilotinib sensitivity. Major cytogenetic response was reached after use of nilotinib, and BCR-ABL^{IS} was reduced to 1.26% at 25 months. In a phase II study, only one out of seven patients with a single mutant Y253H achieved MCgR.[32] Neither of the two patients with E459K achieved MCgR after nilotinib.[32]

Response to tyrosine kinase inhibitor in patients negative for kinase domain mutation

Thirteen patients were negative for the BCR-ABL KD mutation. The two patients with blastic disease (D18, N7) did not respond. All the rest showed CHR after salvage therapy. Major cytogenetic response was achieved in four patients (2 AP, 2 CP), of which two were complete, with one also reaching MMR.

Published studies have found no differences in the overall cytogenetic and molecular responses of patients with and without KD mutations.[32-34] We monitored the responses of our patients on dasatinib 3- to 6-monthly by Taqman RQ-PCR. Reductions in the BCR-ABL transcripts were more readily seen in mutation-positive than mutation-negative patients (Fig). Most mutation-negative patients showed less than 1-log reduction in the BCR-ABL/ABL ratio. The higher dasatinib dose intensity that mutation-positive patients could tolerate as opposed to mutation-negative patients (94.5 vs 51 mg), and the difference in the spectrum of mutations compared to published studies are probably the major reasons for the different responses.

Discussion

In in-vitro studies, T315I mutation is highly resistant to all three TKI agents available in Hong Kong. F317L, V299L, and T315A are mutations that are resistant to dasatinib, and P-loop (E255V/K, Y253H/F) and F359V mutations are resistant to nilotinib.[20,35] In the management of imatinib resistance, the differential response of KD mutations to TKI can guide the clinical choice of alternative TKI agents or other therapies. Our experience and that of others support that F359V mutation predicts good responses to dasatinib even after nilotinib failure.[36] The presence of the highly resistant T315I mutant predicts TKI failure, and allogenic BMT is recommended for suitable candidates. Patients with moderately drug-resistant or resistant mutants may still be responsive to TKI agents, provided that a high enough dose level can be maintained. This is exemplified in our patients with E255K. The cytogenetic responder tolerated a higher standard dose of dasatinib than did the two non-cytogenetic responders. Using the IC_{90} value as a benchmark of clinical benefit, in-vitro studies projected that nilotinib at a trough level of 500 nmol/L would fail to suppress Y253H, E255V, and T315I mutants, while a trough level of 1.5 μmol/L (achievable by nilotinib 400 mg twice daily in pharmacokinetic studies) should suppress all mutants except T315I.[20,35] Dasatinib at a trough level of 50 nmol/L can also suppress all mutants except T315I.[20,35]

There are studies supporting the superiority of dasatinib over nilotinib for controlling disease with P-loop mutants. In phase II studies, the CCgR rates after dasatinib were 40% for E255K/V and 69% for Y253H/F.[37] None of the 13 patients with these mutants reached CCgR after nilotinib.[32] In a study that monitored the dynamics of KD mutation after sequential TKI therapy, new mutations involving P-loop, codons 311 and 359 accounted for more than half of the new mutations after nilotinib.[21] On the other hand, F317L was the most prevalent emerging mutation after dasatinib therapy.[21] As mutational patterns can change during TKI therapy, a repeated

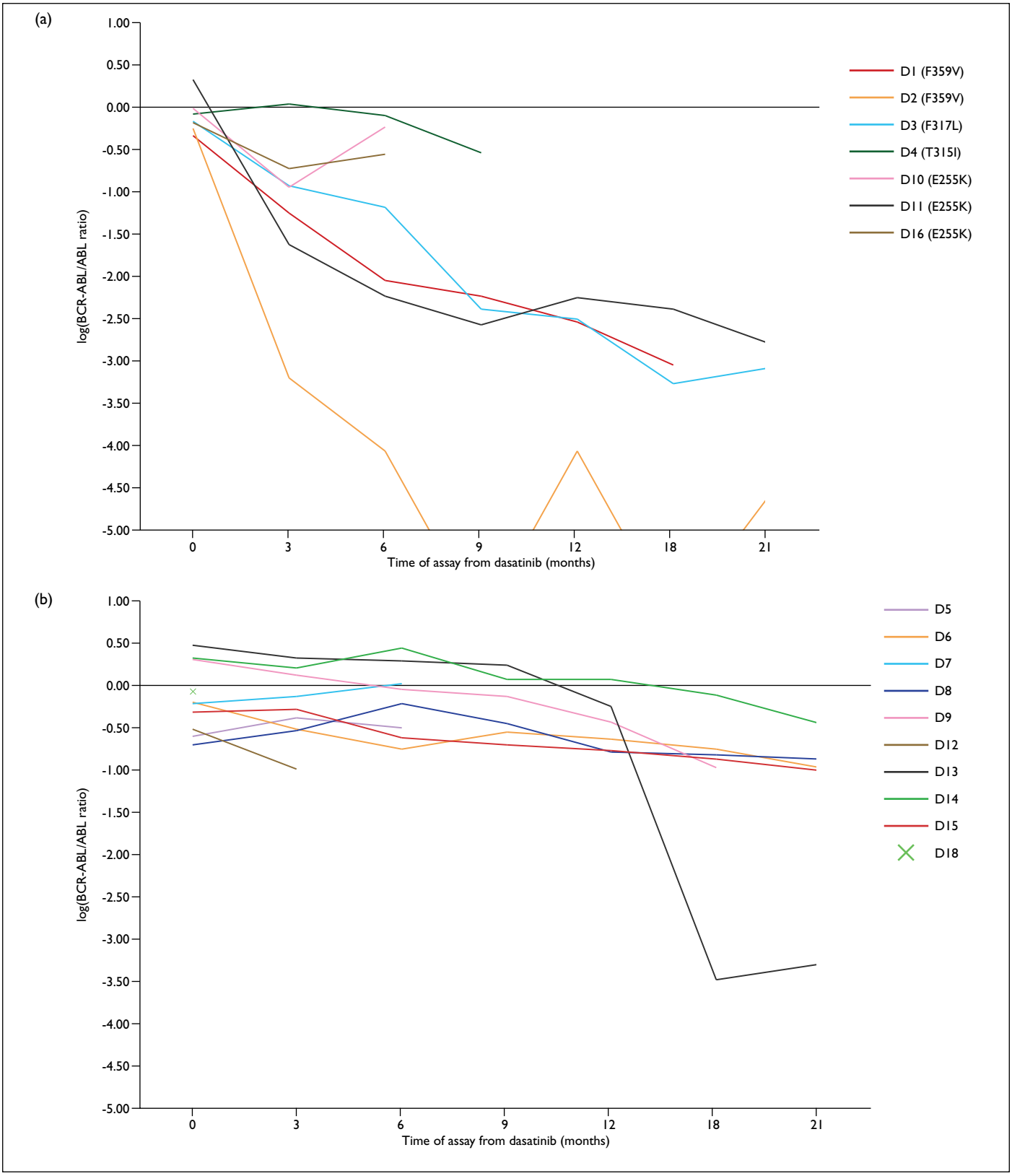

FIG. Three- and six-monthly BCR-ABL/ABL ratios were monitored by real-time quantitative polymerase chain reaction in 17 patients on dasatinib. Results are shown as a log BCR-ABL/ABL ratio plotted against time in (a) mutation-positive and (b) mutation-negative patients

KD mutational analysis is recommended whenever the response is suboptimal. That was done for patient N2 and we noted the switching of the mutational pattern from M351T and G250E to E255V associated with nilotinib failure. Patients with emerging P-loop mutants resistant to nilotinib may be responsive to

dasatinib.[36]

The use of KD mutational patterns to predict responses remains imperfect. In particular, predicting responses and selecting drugs for patients harbouring multiple mutations is not straightforward. Moreover, discordance between the in-vitro drug sensitivity and the in-vivo response is not uncommon. In published studies, patients with the F317L mutation responded poorly to dasatinib.[30] Nilotinib is the preferred agent in this circumstance.[31] The unexpected response to dasatinib in our patient with F317L suggests that the BCR-ABL independent pathway has a functional role in the modulation of drug resistance or discordance. Blastic disease patients had the worst response to TKI agents regardless of their mutational status. This may reflect the more prominent or dominating effect of BCR-ABL independent mechanisms in the progression of advanced leukaemia.[38]

Lastly, patients negative for KD mutation do not provide a 'handle' for response prediction. There are no available data suggesting that one agent is superior to the others for treating non-blastic disease with no KD mutation. Therefore, the drug choice becomes arbitrary and depends more on the relative comparisons of drug costs, dosage tolerated, and side-effects. A more comprehensive profile of the operating mechanisms of TKI resistance and their magnitude in individual patients may reveal more precise and relevant targets amenable to more effective salvage therapy in the future.

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