Outcome of patients with chronic myeloid leukemia with multiple ABL1 kinase domain mutations receiving tyrosine kinase inhibitor therapy

Alfonso Quintás-Cardama, Hagop Kantarjian, Susan O'Brien, Elias Jabbour, Gautam Borthakur, Farhad Ravandi, Srdan Verstovsek, Jianqin Shan, and Jorge Cortes

Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

ABSTRACT

We investigated the impact of carrying more than one *BCR-ABL1* mutation in 207 patients with chronic myeloid leukemia (102 chronic, 61 accelerated, and 44 blast phase) post-imatinib failure. Seven (8%) of 92 patients carrying mutations had more than one mutation: 4 (4%) in chronic phase, 2 (2%) in accelerated phase, and one (1 %) in blast phase. The cytogenetic response rate to second generation TKIs for patients with no, one, or more than one mutation was 88%, 69%, 50% (*P*=0.03) in chronic phase, 54%, 42%, 50% in accelerated phase (*P*=0.67), and 35%, 25%, 0% (*P*=0.63) in blast phase, respectively. No differences were observed in event free survival or overall survival in accelerated or blast phase according to their mutational status. However, the 4-year event free survival rates among patients in chronic phase with no, one, or more than one *BCR-ABL1* mutation were 56%, 49%, and 0%, respectively (*P*=0.02), with overall survival rates of 91%, 69%, and 75%, respectively (*P*=0.13). In conclusion, patients with more than one *BCR-ABL1* mutation fare worse than those with no or one mutation.

Key words: CML, tyrosine kinase inhibitor, ABL1 mutation, multiple mutations

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Introduction

BCR-ABL1 kinase domain mutations represent the most frequent mechanism of resistance to tyrosine kinase inhibitor (TKI) therapy, being detected in 40%-50% of imatinib-resistant patients with chronic myeloid leukemia in chronic phase (CML-CP).[1-6] Over 100 *BCR-ABL1* single-point mutations have been reported in patients with imatinib-resistant CML, which confer different levels of TKI resistance.[7] A threonine to isoleucine substitution at the 315 residue (T315I), which forms a key H-bond interaction with TKIs, results in clinical insensitivity to imatinib, nilotinib, and dasatinib.[3,8-13] Other mutations, however, can be inhibited at least to some extent by 2nd generation TKIs (SG-TKIs). The sensitivity of single-point mutations to both nilotinib and dasatinib has been determined *in vitro* and may inform the selection of TKI upon imatinib failure.[14] Patients with CML can acquire more than one *BCR-ABL1* mutation during sequential TKI therapy, which may result in increased oncogenicity compared with each individual mutation.[15] We analyzed the response rates and survival of patients carrying more than one *BCR-ABL1* mutation during sequential TKI therapy.

Design and Methods

Among 293 patients treated at the MD Anderson Cancer Center with SG-TKI after imatinib failure between November 6, 2003 and December 21, 2008, mutation analysis was available in 207 (71%): 102 in chronic phase (CP), 61 in accelerated phase (AP), and 44 in blast phase (BP) (Table 1). The complete kinase domain of the *BCR-ABL1* oncogene was analyzed using seminested reverse transcriptase PCR followed by direct sequencing.[16] The study was conducted in compliance with the MD Anderson Cancer Center Institutional Review Board.

Results and Discussion

The complete cytogenetic response (CCyR) rate with imatinib among patients in chronic, accelerated or blast phase was 35%, 19%, and 30%, respectively. *BCR-ABL1* mutations upon imatinib failure were detected in 92 (44%) patients: 45% in chronic phase, 54% in accelerated phase, and 30% in blast phase. Median time from diagnosis to detection of one mutation in chronic, accelerated, and blast phase was 76 (range 21-264), 67 (range 4-191), and 39 months (range 3-189), respectively. Median time on imatinib for patients with one mutation in chronic, accelerated, and blast phase was 51 (range 14-80), 38 (range 2-63), and 34 months (range 2-56), respectively. Median time from diagnosis to detection of more than one mutation in chronic, accelerated, and blast phase was 81 (range 23-126), 37 (range 14-60), and 161 months, respectively. Median time on imatinib for patients with more than one mutation in chronic, accelerated, and blast phase was 51 (range 22-77), 25 (range 14-37), and 44 months, respectively.

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Correspondence: Jorge Cortes, M.D., UT MD Anderson Cancer Center, Department of Leukemia, Unit 428 1515 Holcombe Blvd. Houston, TX 77030 USA. Phone: international +713.7945783. Fax: international +713.7944297. E-mail: jcortes@mdanderson.org

The most frequent mutations mapped to residues M351 (n=12), G250 (n=12), E255 (n=9), F359 (n=9), and T315I (n=7), comprising 70% of all mutations. Seven (8%) patients had more than one *BCR-ABL1* mutation (*Online Supplementary Table S1*): 4 (4%) in chronic phase (G250E/Y253H, M351T/F317L, T315I/M351T, M351T/F359V), 2 (3%) in accelerated phase (E255K/E459K, G250E/M351T), and one (2%) in blast phase (E255V/M351T).

After a median follow up of 48 months, the complete cytogenetic response (CcyR) rate with imatinib among patients in chronic, accelerated, or blast phase was 35%, 19%, and 30%, respectively. The cytogenetic response rates with SG-TKIs differed among patients with no, one, or more than one mutation (88%, 69%, 50%, respectively, *P*=0.03) in chronic phase, but not in accelerated phase (54%, 42%, 50%, respectively, *P*=0.67), or in blast phase (35%, 25%, 0%, respectively, *P*=0.63) (Table 1). There was a similar but not statistically significant trend for CCyR rates in chronic phase (59%, 55%, 25%, *P*=0.41) and blast phase (23%, 17%, 0%, respectively, *P*=0.80), but not accelerated phase (32%, 32%, 50%, respectively, *P*=0.87) (Table 2). The median duration of cytogenetic response for patients in chronic phase with no, one, or more than one mutation was 22, 36, and 11 months, respectively (*P*=0.22). These data suggest that carrying more than one BCR-ABL1 mutation may hamper the achievement of cytogenetic response and shorten its duration compared to patients with no or only one mutation.

We next evaluated the survival rates of the cohort, both overall (*Online Supplementary Figure S1*) and according to CML phase and mutational status (Table 1). In chronic phase, the number of *BCR-ABL1* mutations segregated prognostically distinct groups. The 4-year event free survival rates among patients in chronic phase with no, one, or more than one *BCR-ABL1* mutation were 56%, 49%, and 0%, respectively (*P*=0.02) whereas the overall survival rates were 91%, 69%, and 75%, respectively (*P*=0.13), indicating that patients with more than one mutation have a significantly worse event free survival compared to those with no or only one *BCR-ABL1* mutation. However, no significant differences were observed regarding event free or overall survival in accelerated phase (4-year: 21% *vs.* 30% *vs.* 0%, *P*=0.81; 54% *vs.* 47% *vs.* 0%, *P*=0.47) or in blast phase (1-year: 19% *vs.* 13% *vs.* not applicable [patient with longest

Table 1. Patients' characteristics and outcomes on tyrosine kinase inhibitor therapy according to the absence or presence of one or more than one *BCR-ABL1* mutation.

Parameter	No mutations	1 Mutation	>1 mutation	*P* value
N. Pts				
CP	56	42	4	0.14
AP	28	31	2	
BP	31	12	1	
Median age, range	52, 18-79	57, 25-82	58, 27-90	0.02
Female (%)	60 (52)	37 (44)	3 (43)	0.46
Median duration CML (months), range	59, 4-253	73, 4-264	69, 14-161	0.03
Prior imatinib therapy				
< 3 years (%)	53	37	29	0.05
> 3 years (%)	47	63	71	
Prior response to imatinib-%				
CP - MCyR	64	50	0	0.03
- CCyR	45	24	0	0.03
AP - MCyR	46	46	50	0.99
- CCyR	25	35	0	0.5
BP - MCyR	44	64	0	0.35
- CCyR	37	27	0	0.65
N. receiving 2nd generation TKI (%)				
Nilotinib	42 (37)	41 (48)	2 (29)	0.06
Dasatinib	37 (32)	33 (39)	3 (43)	
Bosutinib	34 (30)	10 (12)	2 (29)	
INNO-406	2 (2)	1 (1)	0	
Outcomes with 2G-TKI (%) in CP				
a) Cytogenetic response				
- Any	49 (88)	29 (69)	2 (50)	0.03
- CCyR	33 (59)	23 (55)	1 (25)	0.43
b) PFS rate (%) at 2 years	63	59	25	0.02
c) OS rate (%) at 2 years	96	83	75	0.13

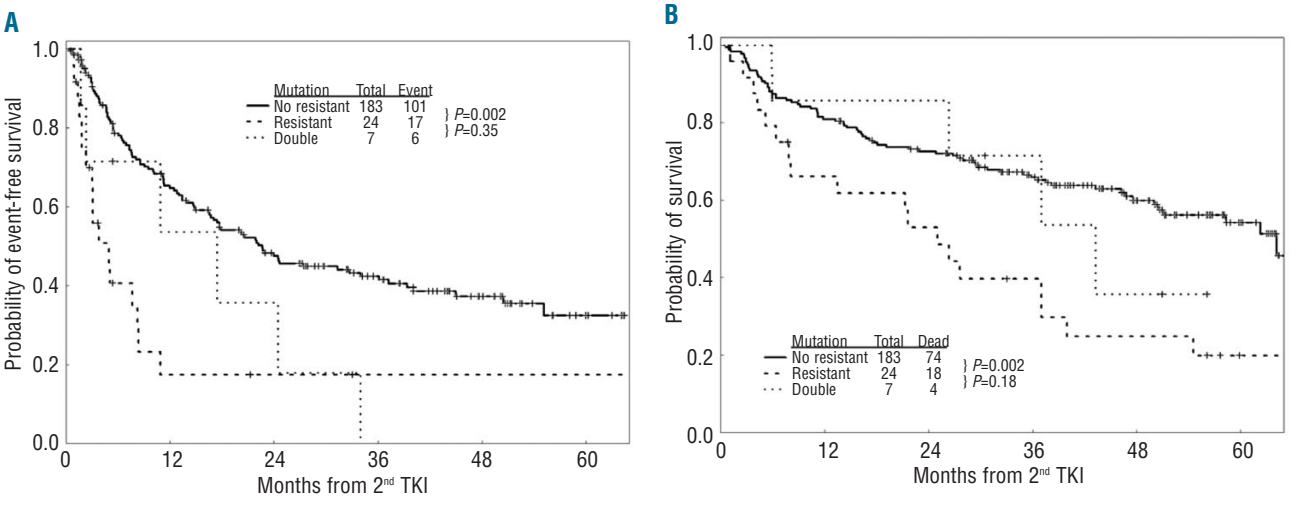

Figure 1. Event-free survival (A) and overall survival (B) of patients with CML-CP carrying none or highly sensitive BCR-ABL1 mutations ("No Resistant") mutations, a single resistant mutation ("Resistant"), or two mutations ("Double").

follow up censored at 5.4 months] *P*=0.24; 1-year: 52% *vs.* 33% *vs.* 100%, *P*=0.39) according to whether they carried no, one, or more than one *BCR-ABL1* mutation, indicating that CML phase bears a higher prognostic weight than mutational status.

M351T was the most frequently detected mutation both alone and concomitantly with other mutations, being present in 5 (71%) of 7 patients carrying more than one *BCR-ABL1* mutation. M351T maps to the activation loop hinge and has lower kinase activity, growth competition potential in low serum, B-lymphoid transformation potency and colony formation capacity compared with native BCR-ABL1 kinase.[17] Importantly, progression free survival and overall survival were similar among patients carrying 2 mutations and those carrying highly resistant single point-mutations (any TKI: T315I; nilotinib: G250E/V, Y253F/H/K, E255K/V, F359C/V; dasatinib: F317L, V299L; bosutinib: E255K/V, F317L, V299L, F486S) (Figure 1A-B). These data suggest that acquiring more than one mutation portends a poor prognosis similar to that associated with the acquisition of a single-point mutation highly resistant to SG-TKIs. Teleologically, the association with a second mutation would allow M351T, a mutation highly sensitive to SG-TKIs, to induce a fully TKI resistant phenotype.

The selective pressure exerted by TKI therapy facilitates the growth of mutant resistant clones. Moreover, acquiring

Table 2. Response according to CML phase, type of 2nd generation tyrosine kinase inhibitor, and the absence or presence of one or more than one BCR-ABL1 mutation

CML phase (inhibitor)	N. mutations at 2ndTKI	N. patients	CCyR	PCyR	Minor CyR	Only CHR	Other	Not Evaluable	% CCyR	Any CyR	% Any CyR	CHR or better	% CHR or better
CP													
	0	56	33	3	13	5	0	2	59	49	88	54	96
	1	42	23	1	5	5	7	1	55	29	69	34	81
	2	4	1	0	1	0	2	0	25	2	50	2	50
	Total	102	57	4	19	10	9	3	56	80	78	90	88
CP-Nilotinib	0	8	4	0	2	2	0	0	50	6	75	8	100
	1	16	8	0	2	2	4	0	50	10	63	12	75
	2	0	0	0	0	0	0	0	0	0	0	0	0
	Total	24	12	0	4	4	4	0	50	16	67	20	83
CP-Dasatinib	0	19	13	0	5	1	0	0	68	18	95	19	100
	1	17	10	0	1	2	3	1	59	11	65	13	76
	2	3	1	0	0	0	2	0	33	1	33	1	33
	Total	39	24	0	6	3	5	1	62	30	77	33	85
CP-Bosutinib	0	27	14	3	6	2	0	2	52	23	85	25	93
	1	8	5	1	1	1	0	0	63	7	88	8	100
	2	1	0	0	1	0	0	0	0	1	100	1	100
	Total	36	19	4	8	3	0	2	53	31	86	34	94
CP-Bafetinib	0	2	2	0	0	0	0	0	100	2	100	2	100
	1	1	0	0	1	0	0	0	0	1	100	1	100
	2	0	0	0	0	0	0	0	0	0	0	0	0
	Total	3	2	0	1	0	0	0	67	3	100	3	100
AP													
	0	28	9	3	3	5	6	2	32	15	54	20	71
	1	31	10	0	3	3	13	2	32	13	42	16	52
	2	2	1	0	0	0	1	0	50	1	50	1	50
	Total	61	20	3	6	8	20	4	33	29	48	37	61
BP													
	0	31	7	1	3	1	15	4	23	11	35	12	39
	1	12	2	1	0	0	9	0	17	3	25	3	25
	2	1	0	0	0	1	0	0	0	0	0	1	100
	Total	**44**	**9**	**2**	**3**	**2**	**24**	**4**	**20**	**14**	**32**	**16**	**36**

one *BCR-ABL1* mutation is associated with an increased risk of acquiring a second mutation.[18] During SG-TKI therapy, imatinib-resistant mutations may associate with newly acquired ones either within the same or in different clones. As patients fail sequential TKI therapy (imatinib followed by an SG-TKI), new mutant clones are selected and may coexist with other previously selected mutant clones. Acquiring new mutations is frequently associated with loss of response.[18] Since patients with CML-CP carrying more than one *BCR-ABL1* mutation appear to have a worse outcome compared with those carrying no or only one mutation, they might benefit from anti-CML agents with a BCR-ABL1-independent mechanism of action. Alternatively, novel TKIs active against mutations resistant to SG-TKIs, such as ponatinib (AP24534) are highly active *in vitro* against CML cells carrying more than one *BCR-ABL1* mutation and may be particularly suited for this particular clinical scenario.[19]

A limitation of our analysis is that mutational status was assessed by direct sequencing, a technique routinely employed in clinical laboratories that only detects mutations reliably when present in at least 20% of the *BCR-ABL1*-expressing cells.[20] More sensitive techniques might have allowed the detection of additional mutant clones at incipient stages of expansion. For instance, a recent mutational analysis using sensitive cloning techniques showed that among 61 patients with imatinib-resistant CML-CP, 57% of them harbored more than one *BCR-ABL1* kinase domain mutation and that the latter were prone to cluster within the same leukemic clone.[21] However, the clinical impact of these clones representing a small proportion of the overall leukemic population remains unknown.

In conclusion, patients with CML-CP carrying more than one *BCR-ABL1* kinase domain mutation exhibit worse response rates and long-term outcomes with TKI therapy compared with those with no or only one *BCR-ABL1* mutation. A longer follow up of a larger series of patients is warranted to confirm these intriguing observations.

Authorship and Disclosures

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