

Assessment of *BCR-ABL1* Fusion Transcripts and Their Association with Response to Imatinib Treatment in Chronic Myeloid Leukemia Patients

Abstract

Objectives: *BCR-ABL1* fusion transcripts with contrasting data on response to imatinib therapy have been reported from different parts of the world. Hence, the present study aimed to determine the frequencies of transcripts and their association with response to imatinib therapy in chronic myeloid leukemia (CML) patients. **Methods:** A total of 170 (76 follow-up and 94 imatinib-resistant) CML samples were included in the study. *BCR-ABL1* fusion transcripts and expression status were analyzed in all cases using multiplex reverse transcriptase PCyR and real-time PCyR. Sanger sequencing was used for tyrosine kinase domain (TKD) mutation screening in imatinib mesylate-resistant patients. **Results:** Of 170 CML patients, 36.36% showed b2a2, 63.53% had b3a2, and 2.94% had b2a2 + b3a2 isoforms. Mean platelet counts and blasts were significantly lower in b2a2 carriers ($P = 0.0092$; $P \leq 0.0001$). Patients with b2a2 transcript were found to be more in responders group (both hematological and cytogenetic), whereas b3a2 patients were more in partial responders group and death ($P = 0.763$; $P = 0.309$). In follow-up patients, mean baseline *BCR-ABL1* expression levels are significantly higher in b2a2 versus b3a2 carriers ($P = 0.0351$). Of 94 imatinib-resistant patients, 36 (38.29%) had acquired TKD mutations. Among 36 patients, mean *BCR-ABL1* levels are significantly higher in b2a2 and b2a2 + b3a2 group ($P = 0.0002$; $P \leq 0.0001$). TKD mutation frequency was more in b3a2 (61.11%) compared to other types. With respect to follow-up status in 36 patients, 17 patients died while 19 were on imatinib higher doses or 2nd-generation tyrosine kinase inhibitors. Of 17 patients, 41.66% had b2a2 transcript and 54.54% had b3a2 transcript. **Conclusion:** Patients with b3a2 transcripts might be associated with poor response and worse prognosis in CML with imatinib treatment.

Keywords: *BCR-ABL1*, chronic myeloid leukemia, fusion transcripts, imatinib, response, tyrosine kinase domain mutations, polymerase chain reaction (PCR)

Sailaja Kagita,
Tulasi Krishna
Mamidi,
Leela Digumarti,
Sadasivudu
Gundeti¹,
Raghunadharao
Digumarti

Department of Medical
Oncology, Homi Bhabha
Cancer Hospital and Research
Centre, Visakhapatnam,
Andhra Pradesh, ¹Department
of Medical Oncology, Nizam's
Institute of Medical Sciences,
Hyderabad, Telangana, India

Introduction

Chronic myeloid leukemia (CML) is a stem cell disorder of myeloid precursors characterized by the presence of Philadelphia chromosome (Ph), observed in 95% of patients. The Ph chromosome is a shortened 22nd chromosome resulting from a reciprocal translocation between the long arms of chromosomes 9 and 22 t(9;22)(q34;q11), leading to *BCR-ABL1* fusion gene with constitutive tyrosine kinase activity. The breakpoint within the *ABL1* gene is almost always at the second exon (a2), while the breakpoint in the *BCR* gene varies and can be localized to one of the three regions: major breakpoint cluster region (M-BCR), minor BCR (m-BCR), and micro-BCR (μ -BCR).^[1] The site of the breakpoint in the *BCR* gene may influence the phenotype of the disease. In majority

of CML cases, the breakpoint almost always in the M-BCR and an abnormal fusion protein p210^{BCR-ABL} (b2a2 and b3a2 isoforms) with enhanced tyrosine kinase activity is formed. Imatinib mesylate (IM) is the first-generation tyrosine kinase inhibitor (TKI) used for treating all Ph-positive CML cases.

Variable frequencies of *BCR-ABL1* fusion transcripts with contrasting data on response rates have been reported from different parts of the world. Earlier studies reported patients with b3a2 transcript had higher survival rates compared to b2a2 transcript.^[2] Sharma *et al.* and Adler *et al.* reported that patients with b3a2 had bad prognosis than patients with b2a2 transcripts and response to imatinib therapy.^[3,4] Hence, the present study aimed to determine the frequencies of *BCR-ABL*

Address for correspondence:
Dr. Raghunadharao Digumarti,
Department of Medical
Oncology, Homi Bhabha Cancer
Hospital and Research Centre,
Visakhapatnam - 530 053,
Andhra Pradesh, India.
E-mail: rdigumarti@gmail.com

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fusion transcripts and their possible association with response to imatinib therapy.

Materials and Methods

A total of 170 CML samples (follow-up: 76 and IM resistant: 94) were included in the study. The study was approved by the institutional ethics committee, and informed consent was obtained from every patient participating in the study. The median age at onset of disease was 40 years (range 6–70 years). Of 170 patients, 109 were males and 61 were females. Maximum of the patients were diagnosed in chronic phase (87.05%) versus acute phase (12.94%).

Six milliliters of the blood sample from each CML patients was collected, and genomic DNA and RNA were extracted using TRIzol method (Invitrogen). The concentration and purity of the RNA were measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). Total RNA (1 µg) was reversely transcribed into complementary DNA using high-capacity reverse transcription kit (Applied Biosystems) and used for of *BCR-ABL1* fusion transcript types^[5,6] and expression analysis.^[7] TKD mutations were analyzed in DNA using Sanger sequencing method.^[8]

BCR-ABL1 fusion transcript analysis

A total of 170 CML samples were analyzed for *BCR-ABL1* p210 and p190 fusion transcripts using multiplex PCR assay.^[5,6] PCR was carried out in a total volume of 20 µL which contains the following: 0.25 µL of primers of each (250 nmol/L) and 5.0 µL of PCR mix (Fermentas), 3.5 µL nuclease-free water, and 2 µL of cDNA. The thermal cycling conditions were as follows: 10 min at 94°C, followed by 35 cycles of 30 s at 95°C, 1 min at 60.4°C, 1.30 min at 72°C, and finally 10 min at 72°C. The PCR products were checked on 2% agarose gel for *BCR-ABL1* fusion transcripts with variable sizes: 481 bp for e1a2, 385 bp for b3a2, 310 bp for b2a2, and 808 bp for normal *BCR* gene.

Statistical analysis

Prognostic scores such as Sokal, Hasford, and European Treatment Outcome Study (EUTOS) were calculated for all patients using baseline hematological variables (<http://bloodref.com/myeloid/cml/sokal-hasford>).

Chi-square test, Student's *t*-test, and ANOVA test were calculated to test the significant association between *BCR-ABL1* transcript types and epidemiological, hematological, and clinical parameters. All the *P* values were two-sided and the level of significance was taken as *P* < 0.05. Statistical analyses were performed using the GraphPad Prism Software, version 6.0 (San Diego, CA, USA).

Results

BCR-ABL1 fusion transcripts were analyzed in 170 CML samples using multiplex qualitative RT-PCR.

Baseline characteristics were represented in Table 1. *BCR-ABL1/ABL* expression levels were analyzed in all 170 cases and TKD mutations in 94 imatinib refractory CML cases. Of 170 cases, 76 (44.70%) were follow-up cases (on standard dose of imatinib 400 mg) and 94 (55.29%) were imatinib-resistant cases (on IM higher doses and 2nd-generation TKIs). Among 170 cases, death occurred in 23 patients (6 in follow-up and 17 in imatinib-resistant group).

There was male preponderance with a male-to-female ratio of 1.78:1. The median age at onset was 40 years with a

Table 1: Baseline characteristics of chronic myeloid leukemia patients

	n (%)
Total cases	
Follow-up	76 (44.70)
Resistant	94 (55.29)
Gender	
Males	109 (64.11)
Females	61 (35.88)
Age at onset (years)	
<30	49 (28.82)
>30	121 (71.11)
Phase	
Chronic	148 (87.05)
Acute	22 (12.94)
Sokal risk	
High	55 (32.35)
Intermediate	50 (29.41)
Low	65 (38.23)
Hasford risk	
High	31 (18.23)
Intermediate	65 (38.23)
Low	74 (43.52)
EUTOS risk	
High	41 (24.11)
Low	129 (75.88)
<i>BCR-ABL1</i> fusion transcripts	
b2a2	60 (36.36)
b3a2	105 (63.63)
b2a2 + b3a2	5 (2.94)
Hematological response at 3 months after Imatinib initiation	
CHR	137 (80.58)
PHR	27 (15.88)
Died	6 (3.52)
Cytogenetic response at 12 months after Imatinib initiation	
CCyR	109 (64.11)
PCyR/NMR	55 (32.35)
Died	6 (3.52)

CHR – Complete hematological response; PHR – Partial hematological response; CCyR – Complete cytogenetic response; PCyR – Partial cytogenetic response; NMR – No molecular response; EUTOS – European Treatment Outcome Study

range of 6–70 years. 71.1% of patients were in >30 years group and 28.82% were younger than 30 years.

With respect to clinical phase, patients were more in chronic phase (87.05%) compared to acute phase (12.94%). Baseline hematological parameters were used to calculate prognostic scores such as Sokal, Hasford, and EUTOS. No difference was observed with respect to risk scores.

Of 170 CML cases, 36.36% (60/170) patients showed b2a2 isoform, 63.63% (105/170) had b3a2, and 2.94% (5/170) had coexpression of both isoforms, respectively.

When response to imatinib was considered, with respect to hematological response at 3 months after initiation of imatinib, 80.58% patients achieved complete hematological response (CHR), 15.88% showed partial hematological response (PHR), and death occurred in 3.52% of patients. With respect to cytogenetic response at 12 months, complete cytogenetic response (CCyR) was observed in 64.11%, partial cytogenetic response (PCyR) in 32.35%, and death in 3.52% of patients [Table 1].

BCR-ABL1 transcripts versus epidemiological parameters

Epidemiological parameters such as gender and age at onset of disease were correlated with *BCR-ABL1* fusion transcripts. When gender was compared with fusion types, b3a2 type was found to be more in males (66.05%) compared to females (54.09%) ($P = 0.061$). No significant association was observed with respect to age and fusion transcript types ($P = 0.790$) [Table 2].

BCR-ABL1 transcripts versus hematological parameters

Hematological parameters such as total leukocyte count (TLC), platelet count (PC), and peripheral blasts were correlated with *BCR-ABL1* fusion transcripts. Patients with b2a2 fusion type had lower TLCs, whereas patients with b3a2 type and b2a2 + b3a2 type had higher TLCs ($P = 0.062$). There was a significant association with PC and fusion types. PCs were found to be lower in patients with b2a2 and b2a2 + b3a2 type fusion carriers compared to b3a2 type carriers ($P = 0.042$). No association was observed with peripheral blasts and fusion types ($P = 0.367$) [Table 3]. Mean PC and blasts were significantly lower in b2a2 type carriers compared to b3a2 or b2a2 + b3a2 types ($P = 0.0092$; $P \leq 0.0001$) [Table 3a].

BCR-ABL1 transcripts versus clinical parameters

With respect to clinical phase, b3a2 carriers were observed to be more in chronic phase and b2a2 + b3a2 carriers in acute phase compared to b2a2 carriers ($P = 0.006$). No significant association was observed with either of the risk scores - Sokal risk or Hasford risk or EUTOS risk ($P = 0.134$; $P = 0.544$; $P = 0.701$) and fusion types.

When hematological response at 3 months after initiation of imatinib was considered, 37.22% of b2a2 patients had

Table 2: Fusion transcripts versus epidemiological variables

	b2a2	b3a2	b2a2 + b3a2	P
Total cases, n (%)				
Follow-up (n=76)	28 (36.84)	48 (63.15)		0.123
Resistant (n=94)	32 (35.95)	57 (60.63)	5 (5.31)	
Gender, n (%)				
Males (n=109)	36 (33.02)	72 (66.05)	1 (0.9)	0.061
Females (n=61)	24 (39.3)	33 (54.09)	4 (6.55)	
Age at onset, n (%)				
<30 years (n=49)	16 (32.65)	32 (65.30)	1 (2.04)	0.790
>30 years (n=121)	44 (36.36)	73 (60.33)	4 (3.30)	

Table 3: Fusion transcripts versus hematological variables

Total cases	b2a2	b3a2	b2a2 + b3a2	P
TLC, n (%)				
<1 lakh/mm ³ (n=68)	29 (42.64)	39 (57.35)	0	0.062
>1 lakh/mm ³ (n=102)	31 (30.39)	66 (64.70)	5 (4.90)	
Platelet count, n (%)				
<4 lakh cu.mm (n=108)	43 (39.81)	60 (55.55)	5 (4.62)	0.042*
>4 lakh cu.mm (n=62)	17 (27.41)	45 (72.58)	0	
Peripheral blasts, n (%)				
<5% (n=127)	43 (33.85)	79 (62.20)	5 (3.93)	0.367
>5% (n=43)	17 (39.53)	26 (60.55)	0	

* - significant. TLC – Total leukocyte count

CHR, 29.62% had PHR, and 16.66% died. However, b3a2 fusion-type patients were found to be more in partial responders group (66.66%) and death (83.33%) versus complete hematological responders (59.85%) group ($P = 0.763$). With respect to cytogenetic response at 12 months after imatinib initiation, 40.36% of b2a2 patients had CCyR, 27.27% had PCyR, and 16.66% had death. However, b3a2 fusion-type carriers had greater PCyR (70.90%) or death (83.33%) than CCyR (55.96%) group ($P = 0.309$) [Table 4].

BCR-ABL1 transcripts and its expression in follow-up cases

In patients on follow-up ($n = 76$), b2a2 type carriers had higher mean baseline *BCR-ABL1* expression levels compared to b3a2 fusion-type carriers ($P = 0.0351$) [Table 5]. When the present status was considered in follow-up cases, six patients died and rest all 70 patients were on imatinib standard dose 400 mg. Of six died cases, five patients carried b3a2 transcript and one had b2a2 transcript.

BCR-ABL1 transcripts and its expression versus tyrosine kinase domain mutations in imatinib-resistant patients

No association was observed between fusion types and *BCR-ABL1* expression at relapse among imatinib-resistant cases ($P = 0.358$) [Table 5]. Of 94 IM-resistant cases,

Table 3a: Fusion transcripts versus mean hematological variables

	Mean±SD			P
	b2a2	b3a2	b2a2 + b3a2	
TLC (lakh/mm ³)	153,750±125,878	1,566,550±91,601	148,960±86,650	0.757
Platelet count (cu.mm)	3.552±1.767	3.75±2.613	3.10±1.063	0.0092*
Peripheral blasts (%)	3.0±2.93	6.203±5.77	1.50±0.957	<0.0001*

* - significant. TLC – Total leukocyte count; SD – Standard deviation

Table 4: Fusion transcripts versus clinical variables

Total cases	b2a2	b3a2	b2a2 + b3a2	P
Phase, n (%)				
Chronic (n=148)	53 (35.81)	93 (62.3)	2 (1.35)	0.006*
Acute (n=22)	7 (31.81)	12 (54.54)	3 (13.63)	
Sokal risk, n (%)				
High (n=55)	20 (36.36)	34 (61.81)	1 (1.81)	0.134
Intermediate (n=50)	18 (36.0)	28 (56.0)	4 (8.0)	
Low (n=65)	22 (33.84)	43 (66.15)	0	
Hasford risk, n (%)				
High (n=31)	10 (32.25)	19 (61.29)	2 (6.45)	0.544
Intermediate (n=65)	26 (40.0)	37 (56.92)	2 (3.07)	
Low (n=74)	24 (32.43)	49 (66.21)	1 (1.34)	
EUTOS risk, n (%)				
High (n=41)	14 (35.0)	25 (65.0)	2 (4.87)	0.701
Low (n=129)	46 (35.65)	80 (62.01)	3 (2.32)	
Hematological response at 3 months after imatinib initiation, n (%)				
CHR (n=137)	51 (37.22)	82 (59.85)	4 (2.91)	0.763
PHR (n=27)	8 (29.62)	18 (66.66)	1 (3.70)	
Died (n=6)	1 (16.66)	5 (83.33)	0	
Cytogenetic response at 12 months after Imatinib initiation, n (%)				
CCyR (n=109)	44 (40.36)	61 (55.96)	4 (3.66)	0.309
PCyR/NMR (n=55)	15 (27.27)	39 (70.90)	1 (1.81)	
Died (n=6)	1 (16.66)	5 (83.33)	0	

* - significant. CHR – Complete hematological response; PHR – Partial hematological response; CCyR – Complete cytogenetic response; PCyR – Partial cytogenetic response; NMR – No molecular response; EUTOS – European Treatment Outcome Study

Table 5: Fusion transcripts and *BCR-ABL1* expression at baseline in follow-up cases versus at relapse in resistant cases

<i>BCR-ABL1</i> expression	Mean±SD			P
	b2a2	b3a2	b2a2 + b3a2	
Follow-up cases	99.52±97.25	71.32±66.69	0	0.0351*
Resistant cases	36.80±33.56	34.0±33.92	56.77±36.44	0.358

* - significant. SD – Standard deviation

36 (38.29%) patients had acquired mutations and presented higher *BCR-ABL1* levels compared to those patients without mutations ($P = 0.0345$) [Table 6].

When fusion types and *BCR-ABL1* expression levels were considered, mean *BCR-ABL1* levels were found to be significantly higher in b2a2 and b2a2 + b3a2 patients with the presence of mutations ($P = 0.0002$; $P \leq 0.0001$) compared to those without mutations. However, in b3a2 carriers, patients without mutations had higher expression levels ($P = 0.177$) [Table 6a].

Median duration to acquire TKD mutations was 48 months, with a range of 12–132 months. Among 36 TKD mutation-positive cases, b3a2 fusion transcript type observed in 61.11% (22/36) of patients, b2a2 in 33.33% (12/36), and b2a2/b3a2 in 5.55% (2/36). With respect to follow-up status, of 36 patients, death occurred in 17 cases (54.54% carried b3a2 and 41.66% had b2a2) while 19 were on imatinib higher doses or 2nd-generation TKIs or on a clinical trial ($P = 0.771$) [Table 6b].

Of 36 TKD mutation-positive patients, the main gatekeeper mutation “T315I” was detected in 15 patients. Of these 15 cases, b3a2 transcript type observed in 10 (66.66%) patients among these 9 (75.0%) cases expired and b2a2 transcript type observed in 5 (33.33%) patients among these 3 (25.0%) members expired.

Discussion

Molecular analyses have become mandatory in the current scenario for diagnostic evaluation and monitoring

Table 6: Tyrosine kinase domain mutations versus *BCR-ABL1* expression in imatinib mesylate-resistant patients

TKD mutations	BCR-ABL1 expression		P
	<10%	>10%	
Presence (n=36)	5 (13.88)	31 (86.11)	0.0345*
Absence (n=58)	21 (36.20)	37 (63.79)	

* - significant. TKD – Tyrosine kinase domain; IM – Imatinib mesylate

Table 6a: Tyrosine kinase domain mutations versus transcript type versus *BCR-ABL1* expression in imatinib-resistant cases

Transcript type	TKD mutations and <i>BCR-ABL1</i> expression		P
	Presence (mean±SD)	Absence (mean±SD)	
b2a2	53.22±39.73	20.47±20.01	0.0002*
b3a2	28.35±9.687	75.72±35.52	0.177
b2a2 + b3a2	49.34±35.40	21.18±21.08	<0.0001*

* - significant. TKD – Tyrosine kinase domain; SD – Standard deviation

Table 6b: Presence of tyrosine kinase domain mutations versus follow-up status in imatinib-resistant cases

Follow-up status	Died, n (%)	IM higher doses/second generation TKI, n (%)	P
b2a2 (n=12)	5 (41.66)	7 (58.33)	0.771
b3a2 (n=22)	12 (54.54)	10 (45.45)	
b2a2 + b3a2 (n=2)	0	2 (100.0)	

TKD – Tyrosine kinase domain; IM – Imatinib mesylate

response rates to targeted treatment modalities in CML patients.^[9-13] Cross *et al.* described a multiplex-PCR assay for the identification of *BCR-ABL1* fusion transcripts, which allows rapid, specific, and simultaneous detection of the three *BCR-ABL1* fusion transcripts in patients with CML and acute lymphocytic leukemia.^[5] In the present study, we aimed to find out the frequency of *BCR-ABL1* transcripts and their association with response to imatinib treatment. However, several studies showed controversial reports between the role of fusion transcripts and prognosis.^[2-4,14-17]

In our study, the frequencies of b2a2, b3a2, and b2a2 + b3a2 transcripts observed to be 36.36%, 63.53%, and 2.94%, respectively. Anand *et al.* found the incidence of b2a2, b3a2, and b2a2 + b3a2 transcripts to be 28.84%, 66.82%, and 3.36%, respectively.^[18] Another study reported the frequencies of b2a2 and b3a2 to be 32% and 68%, respectively.^[19] Deb *et al.* showed the presence b2a2 in 41.25% and b3a2 in 56.25% of CML patients.^[20]

In the present study, males had higher frequency of b3a2 transcript type compared to females ($P = 0.061$). This results are similar to those reported previously.^[19] Other studies by Adler *et al.* and Osman *et al.* reported that males had higher tendency of expressing b2a2 and females a

higher tendency for b3a2.^[4,21]

Patients carrying b3a2 and b2a2 + b3a2 transcript types had slight higher TLCs compared to b2a2 type. Our results are in disagreement with the earlier studies.^[22] We observed significant association with PC and fusion types; PCs were found to be lower in patients with b2a2 and b2a2 + b3a2 type fusion carriers compared to b3a2 type carriers. Our results differ from earlier reports, which reported higher PC in patients carrying b3a2.^[17,23,24]

In our study, we observed a significant number of b3a2 type carriers in chronic phase and b2a2 + b3a2 carriers in acute phase, which are similar to earlier reports.^[18]

We did not find any significant association with either of the prognostic scores. Deb *et al.* showed that the patients with b2a2 variants had higher relative risk according to Sokal and EUTOS score.^[20]

The frequency of CHR and CCyR were found to be more in b2a2 transcript carriers compared to b3a2 or b2a2 + b3a2 carriers, which indicates that b3a2 type patients had poor response to imatinib therapy in the present study. Our results are consistent with earlier reports.^[3,4,17] Earlier studies reported that b2a2 patients had better molecular response compared to b3a2 patients.^[16,25] However, Jain *et al.* reported that patients with b3a2 (alone or with coexpressed b2a2) had earlier and deeper responses, with longer event-free and transformation-free survival.^[26] Another study by Rashid *et al.* did not find any significant difference between transcript types and prognosis.^[27]

In follow-up cases, elevated mean baseline *BCR-ABL1* expression levels were observed in b2a2 patients compared to b3a2 patients, whereas in imatinib-resistant cases, we could not find any significant association in between fusion types. Our results are in disagreement with earlier reports by Sharma *et al.*, who reported high expression levels in b3a2 patients.^[3]

TKD mutation analysis was done in imatinib refractory patients; among 94 patients, 36 (38.29%) cases had acquired mutations with higher *BCR-ABL1* expression levels. The incidence of mutations was found to be more in b3a2 patients compared to b2a2 and b2a2 + b3a2 group of patients. Mean *BCR-ABL1* expression levels are higher in b2a2 and b2a2 + b3a2 type carriers versus b3a2 carriers.

With respect to follow-up status, among 36 TKD mutation-positive patients, the incidence of death was more in b3a2 patients (54.54%) compared to b2a2 patients (41.66%), which indicates that b3a2 carriers had bad prognosis.

There are no previous reports in the literature; this is perhaps the first study, correlating *BCR-ABL1* fusion transcript types with *BCR-ABL1* expression levels and TKD mutations.

Conclusion

In the present study, achievement of CHR and CCyR was found to be more in b2a2 carriers, whereas in b3a2 carriers, TKD mutation frequency was higher and associated with poor survival.

From our study, we can conclude that b3a2 transcripts in CML might be associated with poor response and worse prognosis, when compared to other types (b2a2 or dual expression), with imatinib treatment. The study of *BCR-ABL1* fusion transcript types may help in better prognostication of CML.

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Conflicts of interest

There are no conflicts of interest.

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