

## Original Article

## Frequency of BCR-ABL Fusion Transcripts in Iranian Patients with Chronic Myeloid Leukemia

Marjan Yaghmaie MSc\*, Seyed H. Ghaffari PhD\*, Ardashir Ghavamzadeh MD\*, Kamran Alimoghaddam MD\*, Mohammad Jahani MD\*, Seyed-Asadollah Mousavi MD\*, Masoud Irvani MD\*, Babak Bahar MD\*, Isa Bibordi MD\*

**Background:** A specific chromosomal abnormality, the Philadelphia chromosome, is present in 90 – 95% of patients with chronic myeloid leukemia. The aberration results from a reciprocal translocation of chromosomes 9 and 22, creating a BCR-ABL fusion gene. There are two major forms of the BCR-ABL fusion gene, involving ABL exon 2, but including different exons of BCR gene. The transcript b2a2 or b3a2 codes for a p210 protein. Other fusion gene leads to the expression of an e1a2 transcript, which codes for a p190 protein. Other less common fusion genes are b3a3 or b2a3 (p203) and e19a2 (p230). The incidence of one or other rearrangement in chronic myeloid leukemia patients varies in different reports. In general, fusion transcripts are determined individually, a process which is labor-intensive in order to detect all major fusion transcripts. The objective of this study was to set up a multiplex RT-PCR assay for detection and to determine the frequency of different fusion genes in 75 Iranian patients with chronic myeloid leukemia.

**Methods:** Peripheral blood samples were analyzed by multiplex RT-PCR from 75 adult Iranian chronic myeloid leukemia patients to detect different types of BCR-ABL transcripts of the t(9;22).

**Results:** All patients examined were positive for some type of BCR/ABL rearrangement. The majority of the patients (83%) expressed one of the p210<sup>BCR-ABL</sup> transcripts (b3a2, 62% and b2a2, 20%), while the remaining showed one of the transcripts of b3a3, b2a3, e1a2 or co-expression of b3a2 and b2a2. The rate of co-expression of the b3a2 and b2a2 was 5%.

**Conclusion:** In contrast to other reports, we did not see any co-expression of p210/p190. Co-expression may be due to alternative splicing or to phenotypic variation, with clinical course different from classic chronic myeloid leukemia.

*Archives of Iranian Medicine, Volume 11, Number 3, 2008: 247 – 251.*

**Keywords:** BCR-ABL • chronic myeloid leukemia • multiplex RT-PCR

### Introduction

The hallmark of chronic myeloid leukemia (CML) is the t(9;22)(q34;q11). In this translocation, the 30 segment of the c-abl proto-oncogene on chromosome 9 is juxtaposed with the 50 segment of the bcr gene on chromosome 22.<sup>1-4</sup> Breaks in the c-abl gene typically occur in the first intron. Breaks in bcr

generally occur in one of the three regions—the major breakpoint cluster region (M-bcr), the minor breakpoint cluster region (m-bcr), and the micro-breakpoint cluster region ( $\mu$ -bcr).<sup>5</sup> Breakpoints occurring in M-bcr involve introns 13 or 14 and join exon 13 (also known as b2) or 14 (also known as b3) with exon 2 of abl (a2) resulting in the fusion transcripts b2a2 and b3a2, respectively. These transcripts lead to the production of an 8.5 kb transcript coding for a 210-kDa (p210) chimeric protein.<sup>6,7</sup> Both b3a2 and b2a2 transcripts can be formed as a result of alternative splicing.<sup>8,9</sup> Breakpoints in m-bcr involve the first intron of bcr and join exon 1 (e1) with a2 resulting in a smaller fusion transcript, e1a2, that codes for a 190-kDa (p190) protein.<sup>10</sup> Breakpoints in  $\mu$ -bcr involve

**Authors' affiliation:** \*Hematology-Oncology and Bone Marrow Transplant Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran.

**Corresponding author and reprints:** Seyed H. Ghaffari, Hematology-Oncology and Bone Marrow Transplant Research Center, Shariati Hospital, Tehran, Iran.

Tel: +98-218-490-2638, Fax: +98-218-800-4140,

E-mail: shghaffari2000@yahoo.com

Accepted for publication: 11 July 2007

intron 19 and result in the joining of exon 19 (e19) of bcr with a2, e19a2, coding for a 230-kDa (p230) protein.<sup>11</sup> Although, all bcr-abl fusion proteins display activated tyrosine kinase activity, the p190 form has been shown to have more transforming potential than p210 both *in vitro* and *in vivo*.<sup>12-14</sup> Fusion transcripts b3a2 and b2a2 account for the majority of CML cases. The e1a2 fusion transcript is seen primarily in t(9;22)-positive acute lymphoblastic leukemia and lymphoid blast phase of the CML, but rarely in chronic phase of CML. The rare e19a2 fusion transcript is found in cases of CML with prominent neutrophilic maturation.<sup>15</sup> These unusual neoplasms also have been reported in the literature by some investigators as chronic neutrophilic leukemia.<sup>16</sup>

We conducted this study to set up a multiplex RT-PCR assay for diagnosis of patients with CML at Hematology-Oncology and Bone Marrow Transplant Research Center of Shariati Hospital, and to determine the frequency of different fusion genes rearrangement among Iranian patients with CML.

## Materials and Methods

### Patients and samples

Seventy-five patients with CML who received treatment from June 2004 through October 2005, were enrolled into this study at the time of diagnosis. The diagnosis of CML was established according to clinical presentation and morphologic criteria of bone marrow aspirate, and was confirmed by cytogenetic assay for t(9;22) and multiplex RT-PCR analysis for BCR-ABL transcripts. The median age was 40 (range: 9 – 55) years, 45% were females and 55% were males. Seven patients were in accelerated phase, 12 were in blast crisis, and the remaining were in chronic phase.

### RNA isolation and cDNA synthesis

Mononuclear cells were isolated from 10 – 20 mL of peripheral blood or 0.5 – 1.0 mL of bone marrow aspirate by density gradient centrifugation on Ficol-Hypaque. Total RNA was extracted from almost 10<sup>6</sup> mononuclear cells by TRIZOL (Gibco BRL, Gaithersburg, MD). The integrity of RNA was determined by gel electrophoresis prior to reverse transcription. For cDNA synthesis, the concentration of RNA was first measured by a spectrophotometric method and then the cDNA was synthesized using the first strand cDNA

synthesis kit (Fermentas UAB, Lithuania). One µg RNA was reversely transcribed with 10 U/µL MMLV, in 1x RT buffer, 25 ng/µL random hexamer primer, 25 µM dNTP, 0.01 M DTT, and 2 U/µL RNasin at 75°C for two min, 42°C for one hr, and 75°C for 10 min.

### Multiplex RT-PCR conditions

The cDNA product was amplified with 1 U/µL Taq polymerase, 240 µM dNTP, 1.8 M MgCl<sub>2</sub>, and 0.6 µM of the four primers (CA3, C5e, BCR-C, and B2B). Multiplex RT-PCR assay was performed on a PCR machine with the program of 10 sec at 100°C, one min at 96°C, three min at 60°C, two min at 72°C, 10 sec at 100°C, 20 sec at 97°C, 25 sec at 58°C, 25 sec at 60°C, 10 sec at 78°C, 50 sec at 73°C, and 31 times to step five and 10 min at 73°C. The sequence of oligonucleotide primers used in multiplex RT-PCR for BCR-ABL fusion transcripts as the target gene and BCR transcripts as internal control are shown in Table 1.<sup>5</sup>

In the PCR protocol, cDNA synthesized from K562 cells (b3a2) and from patients with b2a2 cell types were used as positive controls; and sterile water was used as the negative control. Normal BCR transcript was used as the internal control. The PCR products were run on a 2% agarose gel with ethidium bromide to analyze the size of the amplicons.

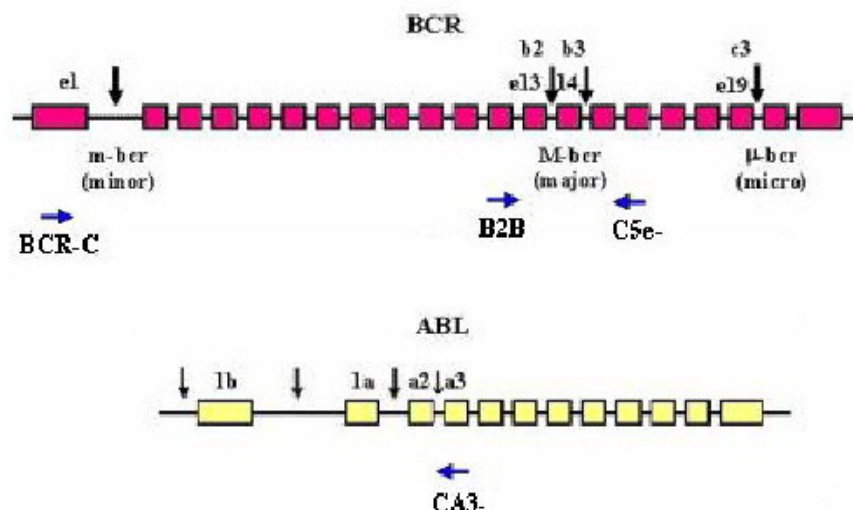
## Results

The primer combinations in multiplex RT-PCR allowed simultaneous detection of all known types of BCR-ABL and BCR transcripts in one reaction (Figure 1). The expected bands were as follows: 808 bp, normal BCR; 481 bp, e1a2; 385 bp, b3a2; 310 bp, b2a2; 209 bp, b3a3; and 103 bp, b2a3.<sup>17,18</sup>

The quality of RNA and efficiency of cDNA synthesis were analyzed by amplification of BCR gene as an internal control. The amplified product (808 bp) from the BCR gene was the only band

**Table 1.** Sequence of oligonucleotide primers used in multiplex RT-PCR for detection of BCR-ABL transcript as the target gene and BCR transcripts as the internal control.

C5e	5' ATAGGATCCTTGGCAACCGGGTCTGAA 3'
B2B	5' ACAGAATTCGGCTGACCATCAATAAG3'
BCR-C	5' ACCGCATGTTCCGGGACAAAAG3'
CA3	5' TGTTGACTGGCGTGATGTAGTTGCTTG G3'



**Figure 1.** Schematic diagram of the position of specific primers on the exons of BCR and ABL used in the multiplex RT-PCR assay for the detection of major fusion transcript in patients with CML.

detected in BCR-ABL- negative patients; absence of this bands indicated procedural failure. The results of multiplex RT-PCR for some different patients are shown in Figure 2.

We were able to reliably detect typical p210 transcripts, such as b2a2 or b3a2, and atypical types, such as transcripts lacking ABL exon a2 (b2a3 and b3a3), or p190<sup>BCR-ABL</sup> transcripts, such as e1a2 in 75 patients at the time of presentation. Figure 3 illustrates the diagram of several BCR-ABL fusion transcripts detected among our patients with their frequency. Using multiplex RT-PCR, the majority of patients (83%) expressed one of the p210<sup>BCR-ABL</sup> rearrangements—b3a2 or b2a2. Table 2 shows clinical data for patients according to their BCR-ABL transcript types. The frequencies of different fusions in pre-bone marrow transplant patients were 63% b3a2, 20% b2a2, 1% e1a2, and 16% rare type fusions. The median age of the patients was over 37.5 years while for patients expressing both b3a2/b2a2, the median age was 25 years.

Usually, one and sometimes two types of mRNA can be expressed in the same patients. For example, one of our patients had both b3a2 and b2a2 fusion genes; another had b3a2, b2a2, and also b3a3 (p203<sup>BCR-ABL</sup>). These cases were diagnosed as “chronic-phase CML,” with cytogenetic abnormalities in addition to t(9;22). In about 9.4% of instances, exon a2 was not expressed; instead exon a3 was expressed which led to b2a3 or b3a3 fusions.

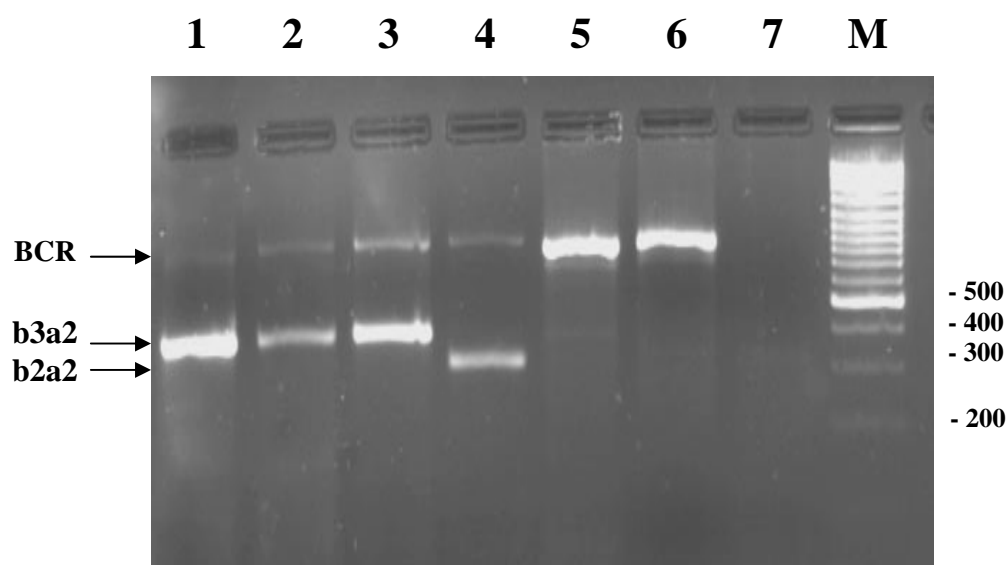
The sensitivity of the assay was evaluated by generating a 10-fold serial dilution series of K562 RNA concentration equivalent to 1 – 10<sup>5</sup> cells in a fixed amount of normal control RNA. Using these

dilutions, as few as one cell in a background of 10<sup>4</sup> normal cells could be detected. The specificity of the assay was evaluated by testing 10 non-CML samples. No detected signal was found with these primer sets. The integrity of the RNA in each sample was confirmed by simultaneous BCR amplification.

## Discussion

RT-PCR assay is a useful tool for the detection of fusion transcript resulting from specific chromosomal translocation of leukemic cells. Multiplex RT-PCR is similar to conventional PCR but includes more than one pair of primers, so that all the known bcr-abl transcripts can be detected. For diagnostic purposes, the use of multiplex PCR has been suggested to detect simultaneously several kinds of BCR-ABL and BCR transcripts as internal controls in one reaction by using three BCR and one ABL primers. Multiplex RT-PCR was successfully developed for the first time in our center for the rapid detection of BCR-ABL transcripts and also to distinguish various BCR-ABL breakpoints in patients with CML. The primer combinations we used in our multiplex PCR give a sensitivity of 10<sup>-3</sup>–10<sup>-4</sup> and can detect all types of fusion transcripts in one PCR reaction. This method allowed us a reliable detection of typical BCR-ABL transcripts such as b2a2 and b3a2, and atypical types such as transcripts lacking ABL exon a2 (b2a3 and b3a3), or transcripts resulting from BCR breakpoints outside the M-bcr, such as e1a2 or e6a2.

BCR-ABL gene rearrangement studies in 75



**Figure 2.** Gel of Multiplex PCR results. M: the 100-bp marker; lane 1, K562 a b3a2-positive cell line; lanes 2 and 3, taken from chronic phase CML patients expressing b3a2; lane 4, taken from chronic phase CML patients expressing b2a2; lane 5, taken from a CML patient in remission which is negative after bone marrow transplantation; lane 6, taken from normal and lane 7 indicates the negative control (water) sample. Lanes 1–7 present the 808-bp band product, as an internal control, taken from the BCR gene and indicate the quality of the RNA and efficiency of the cDNA synthesis.

e1 e14	a2	a11	P210	b3a2	(63%)
e1	a2	a11	P190	e1a2	(1%)
e1 e13	a2	a11	P210	b2a2	(20%)
e1 e13/e14	a3	a11	P203	b3a3/b2a3	(9%)
e1 e13/e14	a2	a11	P210	b3a3/b2a2	(3%)
e1 e19	a2	a11	P230	e19a2	(4%)

**Figure 3.** The diagram of several BCR-ABL fusion transcripts and their frequency in Iranian CML patients.

Iranian CML patients with Philadelphia chromosome showed that the frequency of b2a2 and b3a2 transcripts was 21% and 62%, respectively. In a study by Reiter et al., the incidence of b2a2 and b3a2 transcripts in CML patients with Philadelphia chromosome was 31.6% and 68.4%, respectively.<sup>19</sup> Verschraegen et al., found that the frequency of b2a2 and b3a2 transcripts was 30.2% and 67.9%, respectively.<sup>20</sup> However, in Iranian CML patients, the frequency of b3a2 transcripts was found to be almost three times higher than that of b2a2.

Although relatively few studies are currently available regarding the significance of bcr-abl

transcript type, some preliminary reports suggested that knowledge of transcript type may have clinical application or help us to further understand the pathobiology of t(9;22)-positive leukemic cells. For example, Perego et al.,<sup>21</sup> reported that CML patients with b3a2 transcripts had higher platelet counts than those with b2a2 transcripts. In another study on those with chronic-phase CML, Prejzner<sup>22</sup> suggested that patients with b3a2 transcripts had longer survival than those with b2a2 transcripts. However, we did not observe any significant differences in clinical findings between these breakpoint groups. There was also no difference in the prognosis of patients with b2a2 or

**Table 2.** Patients' clinical data based on BCR-ABL fusion transcript types.

Rearrangement BCR-ABL	Case (%)	Median age (year)	Gender (M/F)	WBC (10 <sup>9</sup> /L)	PLTs (10 <sup>9</sup> /L)
b2a2	15(20%)	32	6/9	200 (17–800)	413 (18–900)
b3a2	47(63 %)	38	30/17	215 (21–775)	481 (100–1200)
b3a3/b2a2	2(3%)	25	2/0	148 (110–207)	467 (364–570)
e1a2	1(1%)	28	1/0	400	700
b3a3/b2a3	7(9%)	42	4/3	399 (340–462)	690 (350–1500)
e19a2	3(4%)	49	2/1	320 (240–400)	865 (840–1090)

b3a2 transcripts.

In this study, we found a low incidence of CML patients (5%) expressing more than one type of mRNA. Co-expression of more than one type of fusion transcript in a patient could be due to alternative splicing or for rare type, due to existence of several leukemic cell lines with different BCR-ABL transcript expression.

## References

- Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973; **243**: 290 – 293.
- de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukemia. *Nature*. 1982; **300**: 765 – 767.
- Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell*. 1984; **36**: 93 – 99.
- Kantarjian HM, Deisseroth A, Kurzrock R, Estrov Z, Talpaz M. Chronic myelogenous leukemia: a concise update. *Blood*. 1993; **82**: 691 – 703.
- Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood*. 1996; **88**: 2375 – 2384.
- Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. *Nature*. 1985; **315**: 550 – 554.
- Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON, Baltimore D. The chronic myelogenous leukemia-specific p210 protein is the product of the bcrabl hybrid gene. *Science*. 1986; **233**: 212 – 214.
- Okamoto K, Karasawa M, Sakai H, Ogura H, Morita K, Naruse T. A novel acute lymphoid leukemia type BCR-ABL transcript in chronic myelogenous leukaemia. *Br J Haematol*. 1997; **96**: 611 – 613.
- Lichty BD, Keating A, Callum J, Yee K, Croxford R, Corpus G, et al. Expression of p210 and p190 BCR-ABL due to alternative splicing in chronic myelogenous leukaemia. *Br J Haematol*. 1998; **103**: 711 – 715.
- Hermans A, Heisterkamp N, von Linden M, van Baal S, Meijer D, van der Plas D, et al. Unique fusion of bcr and c-abl genes in Philadelphia chromosome-positive acute lymphoblastic leukemia. *Cell*. 1987; **51**: 33 – 40.
- Saglio G, Guerrasio A, Rosso C, Zaccaria A, Tassinari A, Serra A, et al. New type of BCR/ABL junction in Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood*. 1990; **76**: 1819 – 1824.
- McLaughlin J, Chianese E, Witte ON. Alternative forms of the BCR-ABL oncogene have quantitatively different potencies for stimulation of immature lymphoid cells. *Mol Cell Biol*. 1989; **9**: 1866 – 1874.
- Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science*. 1990; **247**: 1079 – 1082.
- Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukemia in BCR/ABL transgenic mice. *Nature*. 1990; **344**: 251 – 253.
- Vardiman JW, Imbert M, Pierre R. Chronic myelogenous leukemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. World Health Organization Classification of Tumours. International Agency for Research on Cancer*. IARC Press: Lyon, France; 2001: 20 – 26.
- Pane F, Frigeri F, Sindona M, Luciano L, Ferrara F, Cimino R, et al. Neutrophilic chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood*. 1996; **88**: 2410 – 2414.
- van Rhee F, Lin F, Cross NC, Reid CD, Lakhani AK, Szydlo RM, et al. Detection of residual leukemia more than 10 years after allogeneic bone marrow transplantation for chronic myelogenous leukemia. *Bone Marrow Transplant*. 1994; **14**: 609 – 612.
- Burmeister T, Maurer J, Aivado M, Elmaagacli AH, Grünebach F, Held KR, et al. Quality assurance in RT-PCR-based BCR/ABL diagnostic-results of an interlaboratory test and a standardization approach. *Leukemia*. 2000; **14**: 1850 – 1856.
- Reiter E, Greinix HT, Brugger S, Keil F, Rabitsch W, Mannhalter C, et al. Long-term follow-up after allogeneic stem cell transplantation for chronic myelogenous leukemia. *Bone Marrow Transplant*. 1998; **22** (suppl 14): S86 – S88.
- Verschraegen CF, Kantarjian HM, Hirsch-Ginsberg C, Lee MS, O'Brien S, Rios MB, et al. The breakpoint cluster region site in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. Clinical, laboratory, and prognostic correlations. *Cancer*. 1995; **76**: 992 – 997.
- Perego RA, Costantini M, Cornacchini G, Gargantini L, Bianchi C, Pungolino E, et al. The possible influences of B2A2 and B3A2 BCR/ABL protein structure on thrombopoiesis in chronic myeloid leukemia. *Eur J Cancer*. 2000; **36**: 1395 – 1401.
- Prejzner W. Relationship of the BCR gene breakpoint and the type of BCR/ABL transcript to clinical course, prognostic indexes, and survival in patients with chronic myeloid leukemia. *Med Sci Monit*. 2002; **8**: BR191 – BR197.