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*Full Length Research Paper*

# Unusual location of *BCR/ABL* fusion gene in four CML patients with masked Philadelphia chromosome

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**The author describes atypical *BCR/ABL* fusion patterns among CML patients using FISH technique. The fusion *BCR/ABL* gene is usually observed at the der(22) chromosome while atypical rearrangements in CML patients are rare. As the relocation of *BCR/ABL1* fusion sequence on sites other than 22q11 represents a rare type of variant Ph-translocation, the present study may highlight the hot spots involved in CML pathogenesis and assess their implications in Ph negative *BCR/ABL1* positive CML. This study demonstrates the genetic heterogeneity of this subgroup of CML and the efficiency of FISH in detecting variations of the classical t(9;22). As atypical patterns may have clinical prognostic implications establishment of signal patterns may have important diagnostic implications.**

**Key words:** Variant PH translocations, Ph chromosome, *BCR/ABL*, FISH, chronic myeloid leukemia.

## INTRODUCTION

The t(9;22) translocation, generates the Philadelphia (Ph) chromosome which results in chimeric *BCR/ABL* gene. This gene results from the juxtaposition of the 3' region of the *ABL* oncogene on chromosome 9q34 to the 5' portion of the *BCR* gene on chromosome 22q11.2, in >90% of patients with chronic myeloid leukemia (CML) (Deininger et al., 2000; Goldman et al., 2003). In 5 - 10% of CML cases, there is a variant Ph translocation with generally a third chromosome involved with chromosome 9 and 22 (Zagaria et al., 2004). Cryptic *BCR/ABL* rearrangements can be found in cases with a normal karyotype (Haigh et al., 2004) and in cases with complex karyotype in which the t(9;22) is not detected by conventional cytogenetic analysis. Such rearrangements can be revealed by fluorescence *in situ* hybridization FISH) (Morel et al., 2003). Complex rearrangements in some cases represent secondary changes likely arising (from two consecutive translocations with a total of four breaks, and may arise from multiple simultaneous breaks in some patients, The first translocation resulting in t(9;22) (q34;q11), and the second one involving a break distal to the original one at band 9q34, and another break on a third chromosome (Calabrese et al., 1994). The most frequent location of the *BCR/ABL* fusion gene in complex chromosomal rearrangements is 22q11.2 (Sessarego et al., 2000), but in rare variant cases *BCR/ABL* is translocated on other sites. At least 21 cases described in the literature (Storlazzi et al., 2002; Wan et al., 2004; Fugazza et al., 2005) showed fusion gene located at 9q34. Here we report four CML patients with unusual location of *BCR/ABL* detected with conventional and molecular cytogenetic techniques. The fusion was located on 9q34 in three, of which one showed additional chromosomal anomaly, whereas fourth patient showed novel and complex karyotype with *BCR/ABL* fusion on5q13 (Bakshi et al., 2009).

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**Table 1.** Case history.

| Age/Sex | 13/F | 58/M | 40/F | 35/M |
|---|---|---|---|---|
| Chief complains | Feeling lump in abdomen, weakness on excertion, fever on/off, edema all over body | Fever, history of echymous over right leg and back | Abdominal pain, weakness, low grade fever | Abdominal pain, abdominal fullness |
| Spleenomegaly | + | + | - | + |
| Hemoglobin | 100g/L | 113g/L | 74g/L | 87g/L |
| White blood cell | $172X10^3$ | $165X10^3$ | $86.9X10^3$ | $138X10^3$ |
| Bone marrow (at diagnosis) | CML-CP | CML-CP | CML-CP | CML-CP |
| Treatment | Hydroxyurea (21-Jul-06)/ Imatinib mesylate* (12-Oct-06) | Hydroxyurea (14-Aug-06)/ Imatinib mesylate* (19-Jan-07) | Hydroxyurea (13-Jan-03) | Hydroxyurea (17-Jan-07)/ Imatinib mesylate* (7-Jan-08) |
| Date of diagnosis | 17-Jul-06 | 11-Aug-06 | 8-Jan-03 | 22-Jan-07 |
| Last follow up | 30-Jun-08 | 4-Jul-08 | 9-Jul-08 | 23-Jun-08 |
| Total survival | 24 months | 23 months | 55 months | 18 months |
| Bone marrow (after diagnosis) | CML-CP | CML-CP | CML -CP | CML-CP |
| Hematologic response | Complete | Complete | Complete | Complete |

*(Gleevec;Novartis, East Hanover, NJ.

## MATERIALS AND METHODS

We used two different FISH strategies to study the formation mechanisms of variant Ph translocations in four CML patients. At the diagnosis all the patients were in chronic phase with low level of hemoglobin and higher leukocyte count. Clinical details of each patient are described in Table 1. Cytogenetic studies were performed on bone marrow cells after 16 h culture without mitogenic stimulation. Chromosomal analysis with GTG banding was done and karyotyped according to ISCN 2005 (Shaffer et al., 2005).

To investigate the possible presence of *BCR/ABL,* we undertook FISH studies. Locus Specific Identifier (LSI) dual color dual fusion (DCDF) *BCR/ABL* FISH probe was used initially, this probe mixture contained directly labeled SpectrumOrange™ probe that spanned the *ABL* locus at 9q34 (“O” denotes Orange labeled ABL gene) and directly labeled SpectrumGreen™ probe that spanned the *BCR* locus at 22q11.2 (“G” denotes Green labeled *BCR* gene). The OGYY pattern is the typical pattern for CML and indicates no gross submicroscopic deletions (“Y” denotes Yellow fusion signal of orange and green probe indicative of *BCR-ABL* fusion on derivative 22 and *ABL-BCR* fusion on derivative 9). Atypical patterns of D-FISH include OGGY, OOGY, and OGY which are indicative of deletion of *ABL*, *BCR* and *ABL-BCR* respectively on derivative 9. The LSI *BCR/ABL* Extra Signal (ES) FISH probe was also used to confirm the location of *BCR/ABL* other than 22q11, this probe mixture contained directly labeled SpectrumOrange™ probe that spanned the *ABL* locus at 9q34 (“O” denotes Orange labeled ABL gene) and directly labeled SpectrumGreen™ probe that spanned the *BCR* locus at 22q11.2 (“G” denotes Green labeled *BCR* gene. The OoGY is typical positive pattern, Orange signal of *ABL* gene on normal chromosome#9, small Orange signal of *ABL* gene on derivative chromosome#9, Green signal of *BCR* on normal chromosome#22, and Yellow fusion signal for *BCR/A*BL gene. LSI D75486 7q13 (spectrum orange) with Centromeric Enumeration Probe (CEP) 7 (spectrum green) as internal control was used in patient # 3 to rule out the possibility of isochromosome 7q (Abbott Molecular-Vysis Inc., Des Plaines, IL, USA).

The Multicolor-FISH (M-FISH) was performed in patient #4 to determine the complex chromosomal rearrangements observed in GTG banded metaphases. The manufacturer’s guidelines were followed; slides were pretreated with RNAse and pepsin. Target DNA and M-FISH SpectraVysion probe (Abbott Molecular-Vysis Inc., Des Plaines, IL, USA) were then simultaneously allowed to denature (73°C for 3 min) and hybridize (37°C for 28 h) in Hybrite (Abbott Molecular-Vysis Inc., Des Plaines, IL, USA) instrument. Post-washes were carried out with nonidetP-40 (NP-40) detergent and slides were counterstained with 4,6, diamidino, 2-phenylindole (DAPI).

The analysis of both FISH and M-FISH was done using BX-61 Olympus fluorescence microscope equipped with CCD camera (Jai, Japan) and cytovision (Applied Imaging, UK) software for karyotyping, FISH, and M-FISH.

## RESULTS

### Cytogenetics

In all the patients banding technique did not reveal the Philadelphia chromosome. Patients # 3 and 4 showed other cytogenetic changes. Karyotype descriptions of each patient are as follows:

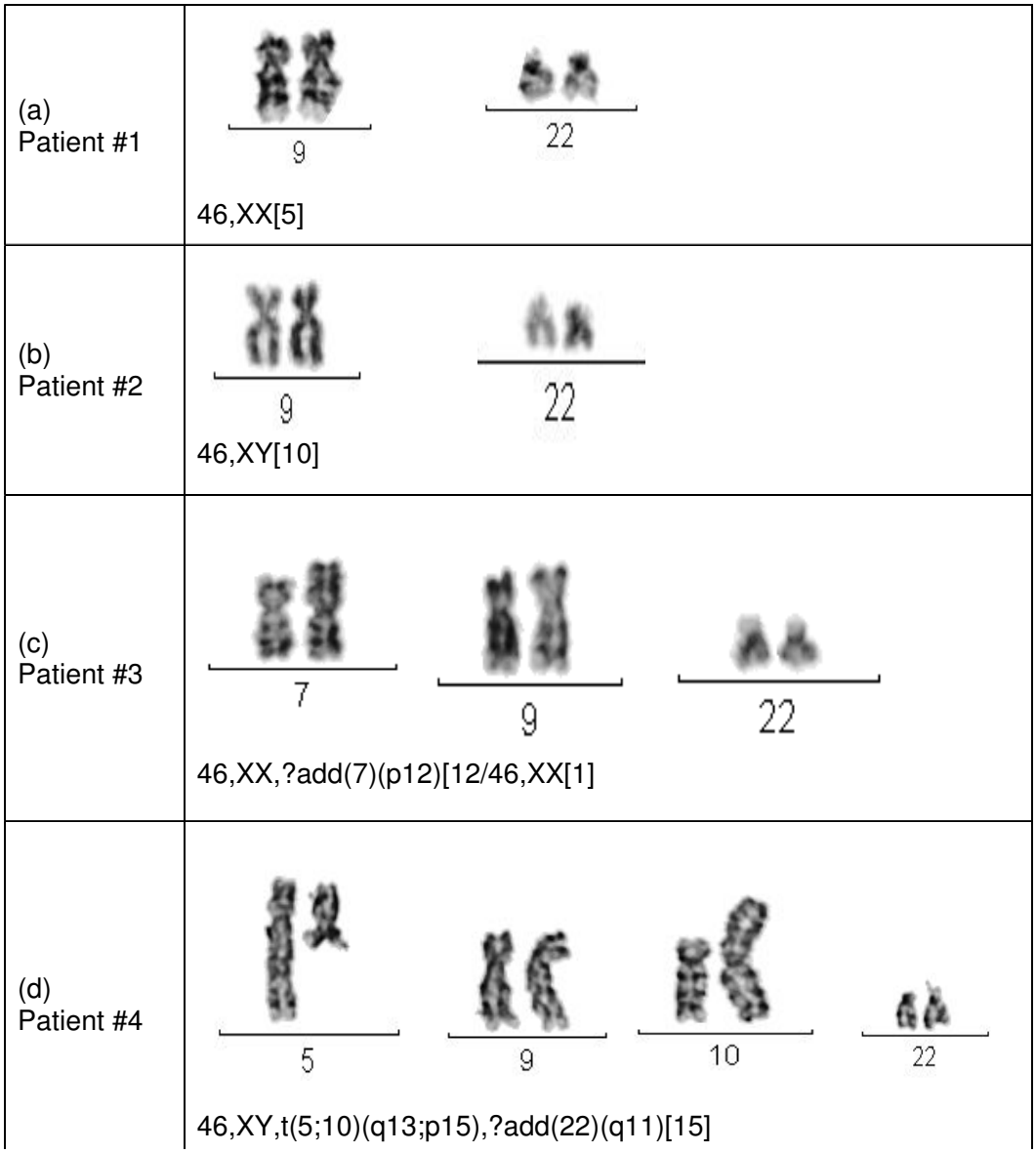


**Figure 1.** (a, b, c, d) Partial GTG banded karyotypes showing masked Philadelphia.

Patient #1: 46, XX [5] (Figure 1a).
Patient #2: 46, XY [10] (Figure1b).
Patient #3:46, XX,? add (7) (p21) [12]/46,XX[1] (Figure1c).
Patient #4: 46,XY,t(5;10)(q13;p?15),?add(22)(q?11)[15] (Figure 1d).

## Fluorescence *in situ* hybridization

### *LSI BCR/ABL dual color dual fusion probe*

Patient # 1 (Figure 2a), 2 (Figure 2b), and 3 (Figure 2c) showed OGGY (Orange signal of *ABL* gene on normal chromosome #9, Green signal of *BCR* on normal chromosome #22, Green signal of *BCR* on derivative chromosome #22,Yellow fusion signal for *BCR/A*BL gene) signal pattern in both interphase and metaphase.

Metaphase FISH showed one orange on normal 9 and one green on normal 22 and one fusion was present on der(9)(q34), whereas only green signal was present on der(22)(q11) instead of second fusion. In patient #4 (Figure 2d), typical positive signal pattern that is, OGYY (Orange signal of *ABL* gene on normal chromosome#9, Green signal of *BCR* on normal chromosome#22, Yellow fusion signal for *ABL/BCR* gene on derivative chromosome #9 and Yellow fusion signal for *BCR/A*BL gene) was observed, but metaphase FISH revealed

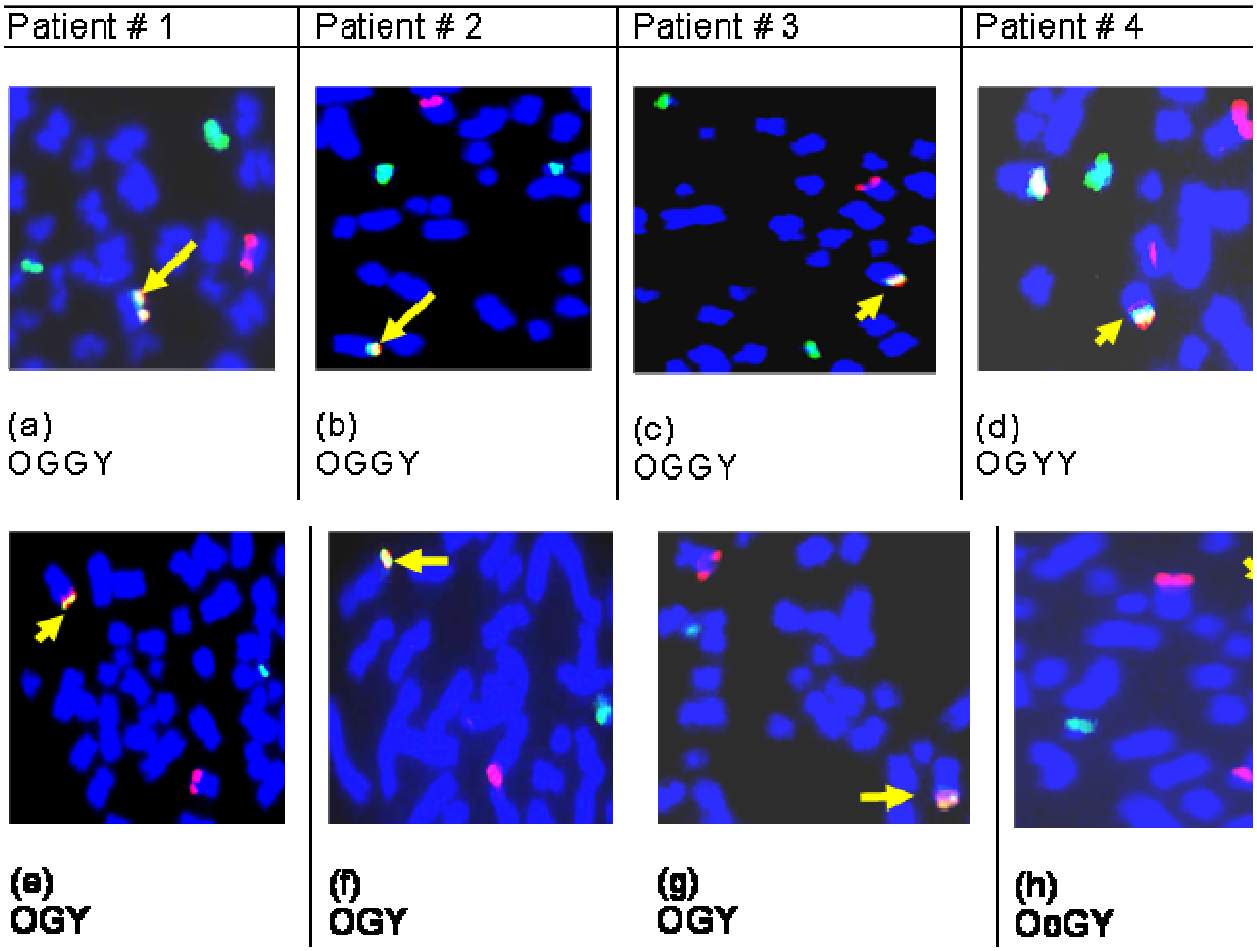


**Figure 2.** Metaphase FISH showing *BCR/ABL* Dual Color Dual Fusion probe results (a, b, c and d) and Extra-Signal probe results (e, f, g, h). Patients #1 (a), #2 (b), #3 (c) showed OGYY signal pattern; Orange signal of ABL gene on normal chromosome#9q; Green signal of BCR on normal chromosome #22q; Green signal of BCR on derivative chromosome #22q; Yellow fusion signal for BCR/ABL gene on der(9q) (Yellow arrow) instead of der(22q). Patient#4 (d) OGYY signal pattern; Orange signal of ABL gene on normal chromosome #9q, Green signal of *BCR* on normal chromosome #22q, Yellow fusion signal for *ABL/BCR* gene on derivative chromosome #9q and Yellow fusion signal for *BCR/ABL* gene on der(5q13) (Yellow arrow) instead of der(22q). Patients #1 (2e), #2 (2f), #3 (g) showed *OGY* signal pattern ;Orange signal of *ABL* gene on normal chromosome#9, Green signal of *BCR* on normal chromosome#22, Yellow fusion signal for *BCR/ABL* gene on der(9q) (Yellow arrow) instead of der(22q). Patient#4 (h) showed OoGY signal pattern; Orange signal of ABL gene on normal chromosome#9q, small Orange signal of ABL gene on derivative chromosome#9q, Green signal of *BCR* on normal chromosome#22q, Yellow fusion signal for *BCR/ABL* gene on der(5q13) (Yellow arrow) instead of der(22q).

second fusion signal for *BCR/A*BL gene on 5q13.

### *LSI BCR/ABL ES (extra signal) probe*

In Patient # 1 (Figure 2e), 2 (Figure 2f), and 3 (Figure 2g), OGY (Orange signal of *ABL* gene on normal chromosome #9, Green signal of *BCR* on normal chromosome #22, Yellow fusion signal for *BCR/A*BL gene) signal pattern was observed, metaphase FISH confirmed the presence and location of *BCR/ABL* gene fusion on der(9)(q34). The patient #4 (Figure 2h), showed typical positive signal pattern that is, OoGY (Orange signal of *ABL* gene on normal chromosome#9, small Orange signal of *ABL* gene on derivative chromosome#9, Green signal of *BCR* on normal chromosome#22, Yellow fusion signal for *BCR/A*BL gene), but metaphase FISH revealed that fusion for *BCR/ABL* was present on der(5)(q13).

### *LSI D75486 7q13 (Patient# 3)*

By using this probe, additional material of unknown origin was confirmed on chromosome 7 as long p-arm observed, it was not an isochromosome. Orange signal for 7q13 and green signal for centromeric enumeration probe (CEP) are depicted in Figure 3a.

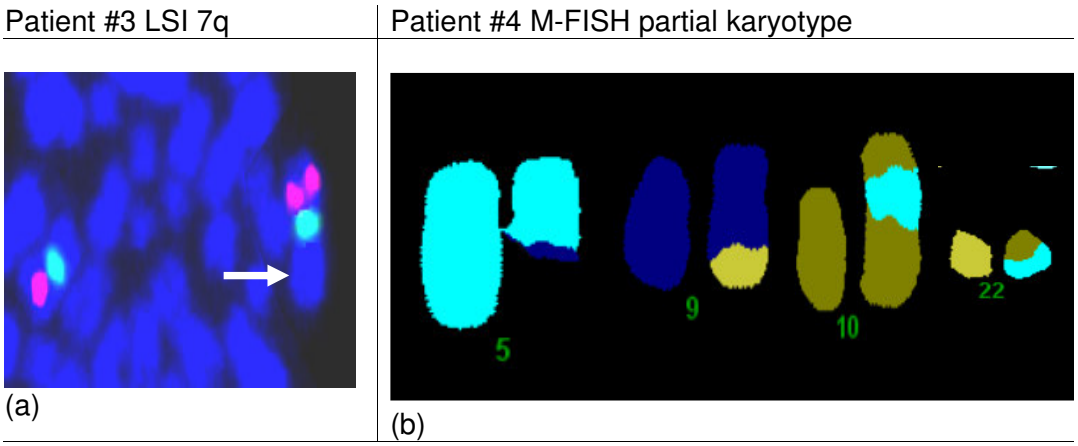


**Figure. 3 (**a) Metaphase FISH is showing addition in 7(p) (white arrow) in patient #3, Orange signal for 7q13 and green signal for Centromeric Enumeration Probe (CEP). (b) M-FISH results showing involvement of four chromosomes (5, 9, 10, 22) in complex rearrangement in patient #4.

***M-FISH (Patient# 4)***

The M-FISH karyotype revealed 46,XY,der(5)t(5;9;22)(q13;q34;q11.2),der(9)t(9;22)(q34;q11.2),der(10)ins(5;10)(q?;p?11),der(22)t(5;22)(?;q11)[5]. The exchange of chromosomal material was seen between chromosomes 5, 9, 10, and 22 as shown in Figure 3b.

## DISCUSSION

Unusual location of *BCR/ABL* gene is a rare event; here we report four such cases. From clinical point of view, the few reported cases with unusual localization of the *BCR/ABL* fusion gene had a poor prognosis (Naumann, 2003; Todoric-Zivanovic, 2006; Gorusu, 2007). Despite the advances made in defining genes related with translocations in CML, the molecular mechanisms of leukemogenesis in the Philadelphia-negative *BCR/ABL* positive CML cases remain to be studied.

Dual color dual fusion FISH (D-FISH) is usually performed to demonstrate the presence of a second fusion signal caused by the chimeric *ABL/BCR* gene on 9q34 (Dewald et al., 2000). In case the signal is absent, it indicates deletion of *ABL* sequences flanking the break-point region. This observation has acquired important prognostic significance (Huntly, 2001). The deletion of residual 5' *ABL* was not confirmed in patients #1, 2 and 3 with masked Ph, as *BCR/ABL* signal mapped exactly on 9q34 instead of 22q11.2 hence, the possible prognostic impact of del9q was not possible to rule out. Our patients showed lower hemoglobin levels and higher leukocyte count. However, neither hemoglobin level nor leukocyte count contributes useful prognostic information in CML (Yoong et al., 2005). The unusual location of *BCR/ABL* sequences on chromosome other than 22q11 apparently did not affect response to Imatinib mesylate as revealed by complete hematologic response (Table 1).

In patient #4 novel complex chromosomal rearrangement involving chromosomes 5, 9, 10, and 22 with the *BCR/ABL* fusion gene translocated on 5q13 was observed, which is not reported before, although variant Ph cases involving 5q13 have been reported in ten cases showing three-way translocation (Mitelman, 2000). Sessarego et al (1988) have reported fragile site at 5q13 and suggested that the presence of this site influenced the involvement of chromosome#5 in the formation of the Ph variant (Sessarego, 1988). The patient #3 did not show any clinical difference as compared to other three patients; still the unusual location of *BCR/ABL* may lead to juxtaposition of genes on the host-site leading to alteration in open reading frames. These areas can be fine-mapped for genomic regions disrupted due to translocation. Proteomics studies can be combined in these cases to see if and how tyrosine kinase is altered.

In conclusion, we report variant localization of *BCR/ABL* other than the normal 22q11. The study also demonstrates crucial role of FISH analysis in disclosing masked Ph chromosome, which is beyond the resolution power of conventional cytogenetics. These observations again underline the importance of metaphase FISH to avoid erroneous interpretation of interphase FISH only. This study also highlights the benefit of combining conventional cytogenetic and FISH analysis for the management of CML patient. The combined cytogenetic, molecular and clinical studies can bring to light the frequency of this event and correlation with prognosis.

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