Detection of derivative 9 deletion by *BCR-ABL* fluorescence in-situ hybridization signal pattern to evaluate treatment response in CML patients

Beena P. Patel[1], Pina J.Trivedi[1], Manisha M. Brahmbhatt[1], Sarju B. Gajjar[1], Ramesh R. Iyer[1], Esha N. Dalal[1], Shilin N. Shukla[2], Pankaj M. Shah[3], Sonal R. Bakshi[1]

SUMMARY

Background: *To evaluate prognostic effect of submicroscopic deletions involving breakage and fusion points of the derivative chromosome 9 and 22 in chronic myeloid leukemia in untreated patients and their follow up samples to correlate with disease outcome.*

Methods: *The study included 78 pretreatment (PT) samples from CML patients and 90 follow-up samples, classified as complete responders (CR, n=33), nonresponders (NR, n =54), and partial responder (PR, n=3) depending on the treatment status of the follow-up samples. Karyotype analysis was performed on metaphases obtained through short term cultures of bone marrow and blood. Detection of BCR–ABL fusion gene was performed using dual color dual fusion (D-FISH) translocation probes.*

Results: *BCR-ABL fusion gene detection by D-FISH showed ABL-BCR deletion on derivative 9 in 47.8% of nonresponders which was higher as compared to pretreatment (11%). Mix D-FISH signal pattern was found in around 20% of pretreatment and non-responder samples. Average interval from chronic phase to blast crisis and accelerated phase was respectively 3.5 and 18 months and accelerated to blast crisis was 16.5 months from the time of diagnosis. The follow-up duration of 31 patients responded to therapy was significantly higher (p=0.0001) as compared to 45 patients who did not respond to therapy. Variant D-FISH signal pattern was seen at the time of diagnosis in patient who responded to therapy as well as those patients who did not respond to therapy.*

Conclusion: *This is the first study from India reporting deletion in ABL, BCR, or ABL-BCR on derivative 9 did not correlate with response to therapy.*

KEY WORDS: *Leukemia, Myelogenous, Chronic, BCR-ABL Positive; Leukemia, Myeloid, Chronic-Phase; Fusion Proteins, bcr-abl; Treatment Outcome; Chromosomes, Human, Pair 9; Chromosome Deletion; Genes, abl; In Situ Hybridization, Fluorescence*

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[1]Cell Biology Division, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad, India, [2]Department of Medical Oncology and Deputy Director Research and Education, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad, India, [3]Department of Medical Oncology and Director, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad, India

Correspondence to:
Dr. Beena P. Patel, Jr. Scientific Officer, Gujarat Cancer Society, Division of Cancer Biology, Cell Biology Division, Room No-302, The Gujarat Cancer and Research Institute, Asarawa, Ahmedabad, India

patel.beena@yahoo.com

Abbreviations:
AP: Accelerated Phase, BAC: Bacterial Artificial Chromosome, BC: Blast Crisis, BCR-ABL fusion gene: Break point Cluster Region-Abelson fusion gene, CML: Chronic Myeloid Leukemia, CP: Chronic Phase, CR: Complete responders, D-FISH: Dual color dual fusion Fluorescence In-situ Hybridization, FISH; Fluorescence In-situ Hybridization, NR: Non responders, PR: Partial response, PT: Pretreatment

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INTRODUCTION

Approximately 5%–10% of chronic myeloid leukemia (CML) patients carry a variant Philadelphia (Ph) chromosome translocation in which the Ph chromosome is derived through rearrangements other than the classic t(9;22). Ph chromosome is formed when the 3' end of the c-ABL proto-oncogene (*ABL*) at 9q34 is relocated to the 5' end of the Breakpoint Cluster Region (*BCR*) gene at 22q11.2 producing the *BCR-ABL* hybrid gene on the derivative 22 and 3' end of BCR is translocated to 9q34 downstream from the 5'*ABL* site, resulting in the reciprocal *ABL-BCR* fusion gene on the derivative 9 (1).

It has been generally thought that patients with classic and variant Ph translocations have the same molecular changes and are clinically and hematologically identical (1). Variant Ph translocations involving several partner chromosomes seem to be more frequently associated with additional chromosomal abnormalities unrelated to the Ph formation (2). It has been reported that *ABL* deletion on derivative 9 was associated with poor prognosis while *BCR* deletion did not affect survival in CML (3,4). However, role of *ABL-BCR* fusion gene is not well documented (5).

A dual color dual fusion Fluorescence In-situ Hybridization (D-FISH) *BCR–ABL* probe system has been designed to span the translocation breakpoints labeled with different fluorochromes to detect deletion in *ABL* or *BCR* or *ABL-BCR* on derivative 9. D-FISH *BCR/ABL* probe is used to monitor residual disease in CML using interphase nuclei as well as it reveals the locations of 3'*ABL* and 5'*BCR* as well as 5'*ABL* and 3'*BCR* on metaphase chromosomes (6). It has been presented at American Society for Clinical Oncology annual meeting in 2007 that deletion in derivative 9 in Chinese patients with CML showed poor prognosis using D-FISH system (7). However, such reports are needed from Asian countries like India to validate the geographic correlation of CML patients with derivative 9 deletions and prognosis of the patients. Therefore the present study aimed to analyze CML patients with deletion in 3'*BCR* and/or 5'*ABL* and/or *ABL-BCR* on derivative 9 to predict disease prognosis in CML patients at the time of diagnosis and during follow-up.

METHODS

Subjects and samples

The study included blood and bone marrow samples from 78 pretreatment (PT) CML patients at the time of diagnosis and their 90 follow-up samples from the medical oncology department of The Gujarat Cancer and Research Institute. Follow-up samples were classified as complete responders (CR, n=33), non responders (NR, n =54) and partial responders (PR, n=3) based on the treatment response. Imatinib was given to all the patients as per the protocol. Clinical details of the patients are given in Table 1.

Table 1. Clinical details of patients and samples

Total number of untreated patients (PT)	n=78
Gender	
Male	n= 51
Female	n= 27
Age: Mean (Range)	39 Yrs. (15-67 Yrs.)
Male: Mean (range)	39 Yrs. (15-67 Yrs.)
Female: Mean (Range)	38 Yrs. (22-65 Yrs.)
CML Classification in PT	
CP	n= 68
AP	n= 08
BC	n= 02
Follow-up samples	n= 90
CR	n= 33
PR	n= 03
NR	n =54
Patients classification according to response	n =78
Patients with complete response	n= 31
Patients with partial response	n= 02
Patient with no response	n= 45
Follow-up duration in months	
Patients with complete response	14 months (2-23 months)
Patients with partial response	11 months
Patient with no response	8.67months (1-25 months)

AP: Accelerated Phase, BC: Blast crisis, CP: Chronic Phase

Study ethics

The study design and patients consent to participate in the study was ethically approved by hospital based ethical committee of the Institute.

Methods

Conventional cytogenetic analysis

Giemsa banded metaphases obtained through short term cultures of bone marrow and/or blood cells using standardized protocols. Karyotypes were reported in accordance with ISCN 2005 (8). Metaphase cells were captured and analyzed using automated karyotyping system consisting of Axioplan universal epifluorescence microscope (Carl Zeiss) and IKAROS software (Metasystems, Germany).

Fluorescence in situ hybridization (FISH)

Detection of BCR–ABL fusion gene was performed using *BCR/ABL* D-FISH translocation probe according to the manufacturer's instructions (Vysis, Downers Grove, Illinois, USA). 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). Around 200 interphase/metaphase nuclei were analyzed for the presence of fusion signals. 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. Complex rearrangement of three way translocation includes signal pattern OOGGY. Image acquisition was performed either on automated Olympus epifluorescence microscope and Cytovision software (version 3.7, Applied Imaging System) or Carl Zeiss with ISIS software (Metasystems, Germany).

Treatment response criteria

Treatment response in follow-up samples was evaluated based on BM pathology/morphology report, and cytogenetic analysis. The CR was defined as follow-up sample showing absence of Ph chromosome and/or absence of *BCR-ABL* fusion gene by FISH and BM morphology report as remission. The NR was defined as presence of Ph chromosome and/or presence of *BCR-ABL* fusion gene by FISH and no morphology change/relapse in BM report. PR was defined as presence of both Ph positive and negative cells and/or presence of positive and negative mixed clone of *BCR-ABL* fusion gene by FISH and BM pathology report being stable as compared to previous report.

Statistical analysis

Data were statistically analyzed using SPSS statistical software (version 13.0; SPSS, Inc., Chicago, IL, USA). Student *t* test was performed to compare follow-up duration between patients responded and not responded to therapy. Pearson correlation test was used to assess association between conventional cytogenetic and D-FISH results. Statistical significance was considered when 'p' values were less than 0.05.

RESULTS

Conventional cytogenetics: Table 2 shows frequency of karyotype in pretreatment and follow-up samples. PT samples showed 43.6% non informative for t(9;22), 53.8% were positive for t(9;22), 1.3% normal karyotype while 1.3% complex karyotype in addition to t(9;22). CR showed 6% non informative and 93.93% normal karyotype. NR showed 20.37% non informative, 74.08% for t(9;22), 1.85% normal, 1.85% complex and 1.85% with mixed clone. PR showed 33.3% each of non informative, t(9;22) and mixed clone by cytogenetics.

Table 2. Frequency of karyotype in pretreatment and follow-up samples

Karyotype	PT (n=78)	Follow-up samples		
		CR (n=33)	NR (n=54)	PR (n=3)
*Noninformative	34 (43.6%)	2 (6.06%)	11 (20.37%)	1(33.3%)
46XX,t(9;22)/46XY,t(9;22)	42 (53.8%)	-	40 (74.08%)	1(33.3%)
46XX/46XY	1(1.3%)	31 (93.93%)	1(1.85%)	-
Complex karyotype involving other chromosome & t(9;22)	1(1.3%)	-	1(1.85%)	-
Mix clone [Normal and t(9;22)]	-	-	1(1.85%)	1(33.3%)

*Noninformative karyotype was defined for poor quality metaphase preparations

D-FISH: Table 3 shows frequency of D-FISH signal pattern in pretreatment and follow-up samples. PT showed 66.7% positive for *BCR-ABL* fusion [OGYY], 11.1% had deletion of *ABL-BCR* [OGY], and 22.2% showed multiple variant signal patterns that are OGY, OOGY, OGGY and complex rearrangement [OOGGY]. CR showed 100% samples negative [OOGG] for *BCR-ABL* fusion gene. NR showed 34.78% positive for BCR-ABL fusion [OGYY], 47.8% deletion *ABL-BCR* [OGY] pattern and 17.39% mixed variant and complex signal patterns showing OGY, OOGY, OGGY and OOGGY. PR showed 100% samples negative for *BCR-ABL* fusion gene [OOGG].

Table 3. Frequency of D-FISH pattern in pretreatment and follow-up samples

D-FISH	PT (n=18)	Follow-up samples CR (n=11)	NR (n=23)	PR (n=2)
Positive (OGYY)	12 (66.7%)	-	8 (34.78%)	-
Negative (OOGG)	-	11 (100%)	-	2 (100%)
Positive (OGY)	2 (11.1%)	-	11 (47.8%)	-
Mix positive variant D-FISH signal pattern (OGY/ OOGY/ OGGY/ OOGGY)	4 (22.2%)		4 (17.39%)	-

Correlation analysis

Pearson correlation analysis was performed to correlate conventional cytogenetics method with that of D-FISH methods. The percentage Ph positive cells by conventional cytogenetics analysis were significantly and positively correlated (r=0.49, p=0.004) with percentage of *BCR-ABL* fusion gene positive cells by D-FISH.

Conventional cytogenetics and D-FISH in patients who responded to therapy

Table 4 shows Karyotype and D-FISH pattern at the time of diagnosis in 9 patients who responded to therapy. Patient no. 19 and 21 were with CML-CP and CML-AP respectively. They showed t(9;22) with no variant D-FISH signal. Patient no. 164, 173, 189, 221 and 287 showed deletion of *ABL-BCR* on derivative 9 in 2%-14% cells. While patient no. 274 and 279 showed deletions of ABL [OGGY] and *ABL-BCR* [OGY] on derivative 9 as well as complex rearrangement [OOGGY].

respectively showing deletion of *ABL-BCR*, *BCR* on derivative 9 [OGY, OOGY] as well as complex rearrangement [OOGGY].

Table 5. Karyotype and D-FISH pattern in pretreatment samples of patients who did not respond to therapy

Pt. ID#.	Age	Sex	CML staging	Karyotype	% OGYY	% OOGG	% variant	variant type
55	57	M	CP	46,XY,t(9;22) (q34;q11.2) [10]	100	0	0	-
110	37	M	CP	46,XY,t(9;22)(q34;q11.2)[12]	98	2	0	-
113	30	M	CP	46,XY,t(9;22)(q34;q11.2)[11]	100	0	0	-
180	32	F	CP	46,XX,t(9;22)(q34;q11.2)[10]	100	0	12.59	OGY
218	30	F	CP	46,XX,t(9;22)(q34;q11.2)[14]	100	0	5.18	OGY
258	45	F	AP	*Noninformative	84.83	2.07	13.10	OGY
260	15	M	CP	46,XY,t(9;22)(q34;q11.2)[20]	23.35	14.38	62.27	OGY+OOGY
292	52	F	CP	*Noninformative	91.55	1.41	7.04	OGY+OOGGY

AP: Accelerated phase, CP: Chronic Phase, *Noninformative karyotype was defined for poor quality metaphase preparations

Follow-up duration in patients who responded and those who did not respond to therapy

Patients responded to therapy showed significantly longer follow-up duration (95% C.I. 2.99-8.77 months, p=0.001) than patients who did not response to therapy (student's *t*-test).

Representative D-FISH pattern of a responder patient

A 55 year old female diagnosed as CML-CP in April 2006. Her BM Blasts were 3%, BM morphology was in accordance with CML-CP. Cytogenetic analysis showed 46,XX, t(9;22) (q34;q11.2). FISH analysis showed 2.1% positive cells (OGYY), 3.4% negative cells (OOGG), and 94.47% cells with variant D-FISH signals which included deletion of *ABL-BCR* in 76.3% cells (OGY), deletion of ABL in 15.67% cells (OGGY) on derivative 9 and complex rearrangement in 2.5% (OOGGY) (Figure 1 A and B). Cytogenetic remission was seen after treatment with Imatinib in April 2007 at 12 months with normal Karyotype. D-FISH results showed 99.65% cells negative for fusion (OOGG) and only 0.35% cells with deletion of *ABL-BCR* fusion on derivative 9 (OGY) (Figure 1 C and D). BM morphology showed CML in remission.

Table 4. Karyotype and D-FISH pattern in pretreatment samples of patients who responded to therapy

Pt. ID #	Age	Sex	CML staging	Karyotype	% OGYY	% OOGG	% variant	Variant type
19	18	M	CP	46,XY,t(9;22)(q34;q11.2)(6)	96	4	0	-
21	37	F	AP	46,XX,t(9;22)(q34;q11.2)[15]	98	2	0	-
164	35	F	CP	46,XX,t(9;22)(q34;q11.2)[10]	94.34	0	5.66	OGY
173	35	F	CP	*Noninformative	86.37	0	13.63	OGY
189	28	F	CP	46,XX,t(9;22)(q34;q11.2)[15]	84.51	2.6	12.89	OGY
221	38	M	CP	*Noninformative	94.29	1.43	4.28	OGY
274	55	F	CP	46,XX,t(9;22)(q34;q11.2)[12]	2.1	3.4	94.49	OGY+OGGY
279	60	F	CP	46,XX,t(9;22)(q34;q11.2)[7]	17.94	1	81.06	OGY,OOGGY
287	55	M	CP	46,XY,t(9;22)(q34;q11.2)[7]/ 45,X,-Y,t(9;22) (q34;q11.2) [3]	96.56	1.15	2.29	OGY

AP: Accelerated phase, CP: Chronic Phase, *Noninformative karyotype was defined for poor quality metaphase preparations

Conventional cytogenetics and D-FISH in patients who did not respond to therapy

Table 5 shows Karyotype and D-FISH pattern at the time of diagnosis in 8 patients who did not respond to therapy. Patient no. 55, 110, and 113 were of CML-CP stage. They showed t(9;22) with no variant D-FISH signal. Patient no. 180, 218, and 258 showed deletion of *ABL-BCR* on derivative 9 in 5-13% cells. Patient no. 260 and 292 showed presence of t(9;22) and non informative by cytogenetic respectively. However, both showed 62% and 7% clones

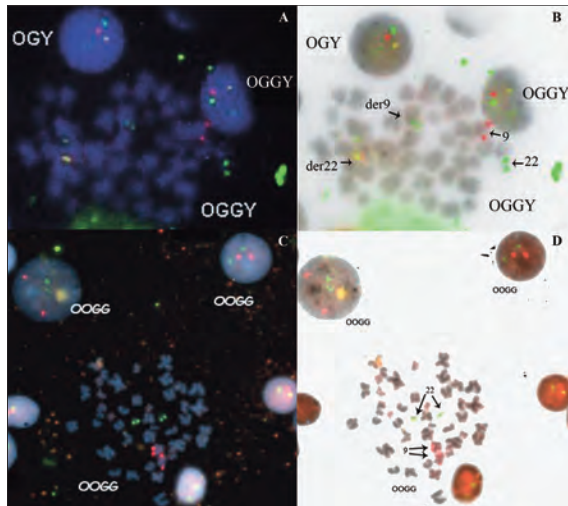

Figure 1. Representative D-FISH signal pattern of a responder patient
(A) Metaphase plate showing OGGY signal pattern at the time of diagnosis. (B) Invert gray scale image of figure 1 A. Normal chromosome 9 and 22 shows orange (O) and green (G) signals. Derivative 22 shows yellow (Y) fusion, while deletion of ABL on derivative 9 shows one green (G) signal. (C) 12 months treated Follow-up sample negative for BCR-ABL fusion gene in interphase and metaphase cell. (D) Invert gray scale image of Figure 1 C. The fluorescent images for Figure 1 A was taken from epifluorescence microscope of Carl Zeiss with ISIS software (Metasystems, Germany), using 63x oil immersion objective (NA=1.4) and CCD camera (progressive scan IλI, Japan). The fluorescent images for Figure 1 C was taken from Olympus epifluorescence microscope and Cytovision software (version 3.7, Applied Imaging System) using 60x oil immersion objective (NA=1.25), using CCD camera (progressive scan IλI, Japan).

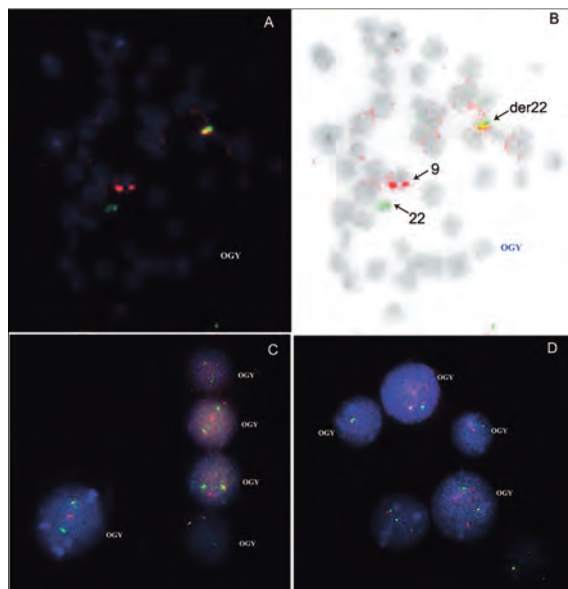

Figure 2. Representative D-FISH signal pattern of a non responder patient
(A) Metaphase plate showing OGY signal pattern after 22 days of diagnosis. (B) Invert gray scale image of figure-2A. Normal chromosome 9 and 22 shows orange (O) and green (G) signals. Derivative 22 shows yellow (Y) fusion. (C-D) Interphase cells showing OGY variant D-FISH signal pattern. The fluorescent images for figure 2 A,C & D were taken from epifluorescence microscope of Carl Zeiss with ISIS software (Metasystems, Germany), using 63x oil immersion objective (NA=1.4) and CCD camera (progressive scan IλI, Japan).

Representative D-FISH pattern of a nonresponder patient

A 37 year old female diagnosed as CML-CP with 6% bone marrow blasts in 1st week of September 2006. However, cytogenetic study showed normal karyotype. After 21 days, D-FISH was performed on fresh BM culture to confirm CML diagnosis for *BCR-ABL* fusion gene. The BM morphology report on 22nd day of follow-up showed 80% blasts with morphological diagnosis of CML-BC. Cytogenetic report was negative for Ph chromosome showing normal karyotype. However, FISH test showed 3.22% cells positive for *BCR-ABL* fusion gene (OGYY), 19% cells negative for fusion gene (OOGG) and 77.42% cells showed deletion of *ABL-BCR* on derivative 9 (OGY) (Figure 2 A-D).

DISCUSSION

The present study aimed to detect whether deletion of *ABL* or *BCR* or *ABL-BCR* on derivative 9 can predict disease prognosis in CML patients at the time of diagnosis and during follow-up and to discusses the present results with other reported controversial observations and suggest the prospect for further research in this direction.

FISH plays a complementary role in providing information in which cytogenetic studies are inadequate because of poor metaphase yield as seen in 43% of PT and 19% NR showing non informative metaphase (9). D-FISH *BCR/ABL* probe used in the present study is useful to reveal the locations of 3' *ABL* and 5'*BCR* as well as 5'*ABL* and 3'*BCR* on metaphase chromosomes making this technique capable of detecting minimal residual disease in CML (10). Present study calculated Pearson correlation analysis for percentage Ph positive cells by conventional cytogenetics and percentage FISH positive cells. Our conventional cytogenetic results are in good agreement with the results of D-FISH analysis as observed by Gouill et al (11). An excellent concordance was found between these two methods for percentage of Ph and FISH positive cells in pretreatment as well as follow-up samples with different treatment response. In the present study, complex variant translocation by karyotype was seen in 1 to 2% of PT and NR. It has been widely accepted that the clinical, prognostic, and hematological features of CML patients with complex variant translocations are not different from those with the classical t(9;22) translocation because pathological event is the formation of the *BCR/ABL* fusion gene (12). D-FISH is useful in two ways; first, when conventional cytogenetic is non informative and second when conventional cytogenetics is informative but it cannot detect submicroscopic deletions on derivative 9. However, D-FISH alone cannot be performed because conventional cytogenetics is important to identify partner chromosome in three way translocation. Therefore ours and others findings suggest that interpretation of results should be made in light with conventional cytogenetic and FISH (10).

Similarly, deletion of *ABL-BCR* (OGY) on derivative 9 was seen in 11.1% of PT which was comparable with reported 15%-20% cases in CML (13-15). While deletion in *ABL*, *BCR* or complex three way translocation (OGGY, OOGY, OOGGY) was seen in 22.2% of PT which is comparable with 24.4% of patients reported by Lee et al (16). Deletion of any of the *BCR* or *ABL* genes on derivative chromosome 9 was reported to be associated with shorter overall and event free survival resulting into poor prognosis (15,16). Patients with *ABL-BCR* deletion represented by OGY variant signal pattern by D-FISH were not different in terms of clinical or laboratory features. This observation has been confirmed by others who also reported shorter chronic phase duration (17), a poor response to alpha interferon therapy (17), and a higher incidence of post bone marrow transplant relapse rate (13). NR showed deletion in *ABL-BCR* in 47.8% indicated by OGY pattern and 17.39% showed variant D-FISH

signal pattern suggesting deletion of *ABL*, *BCR*, *ABL-BCR,* and complex rearrangement [OGGY, OOGY, OGY and OOGGY respectively]. Such variant D-FISH signal pattern was not seen in CR group. In other words, frequency of deletion in derivative 9 was higher in NR (47.8%) as compared to PT (11.1%) group, while mixed variant D-FISH signal pattern was comparable between the two groups (22.2% in PT and 17.39% in NR).

These observations prompted us to compare conventional cytogenetic and D-FISH results obtained at the time of diagnosis between the group of patients who responded to the therapy with the group who did not respond to therapy (Table 4 and 5). Nine patients who responded to therapy and 8 patients who did not respond to therapy showed similar percentage and type of cells with variant D-FISH signal pattern at the time of diagnosis. In fact two patients who responded to therapy showed highest percentage of variant D-FISH signals pattern (80 and 94%, Table 4) as compared to those patient who did not respond to therapy (62%, Table 5). Therefore deletion in *ABL*, *BCR,* or *ABL-BCR* on derivative 9 was not associated with response to therapy as seen in a representative case of responder diagnosed with CML-CP showing t(9;22) and 76% cells with *ABL-BCR* deletion by D-FISH (Figure 1 A and B). After 12 months patient's karyotype was 46,XX and D-FISH result was 99.6% of the cells negative for the fusion gene (OOGG, Figure 1 C and D). Our patients who responded to the therapy showed controversial results (16) showing significantly longer follow-up duration as compared to patients who did not respond to therapy (95% C.I. 2.99-8.77 months, $p=0.001$). In this study we found that even 1% of cells with *BCR-ABL* fusion gene was detected using D-FISH probe. This is in accordance with the earlier report of 1% minimal residual disease detection limit of D-FISH probe (17).

It has been reported that deletion in derivative 9 is associated with shorter chronic phase (13,14,18). A representative case as mentioned in figure-2 was diagnosed as CML-CP, D-FISH was performed on 22nd day of the initial diagnosis. The BM morphology showed blast crisis with 80% blasts with D-FISH showed *ABL-BCR* deletion (OGY) in derivative 9 in 77.4% cells. However, it is not known why the time interval from chronic phase to blast crisis varies among CML patients (19). We have observed that average interval from chronic phase to blast crisis is 3.5 months, accelerated to blast crisis is 16.5 months and chronic to accelerated phase is 18 months from the date of diagnosis.

In this study deletion on derivative 9 was heterogeneous involving either *ABL* or *BCR*, or *ABL-BCR*. This raises the question which region of the derivative 9 is important for disease prognosis. Many tumor-related genes are located near the translocation breakpoints (14). It has been reported that p21rac acts on cell growth and proliferation associated with *RAS*, which moves along with 3'*BCR* region on derivative 9 during translocation. GTPase-activating protein binds with p21rac and inhibits its activity. Therefore, a loss of this region can induce abnormal cell growth and proliferation (20). If these genes are deleted during *BCR-ABL* gene rearrangement and the residue allele is injured by "two hit" events, the tumor suppressor functions are destroyed and the disease can progress. Similarly argininosuccinate synthetase (*ASS*) gene located on 9q34 region adjacent to *ABL* and Immunoglobulin light chain (*IGLL1*) gene located near *BCR* gene on chromosome 22 might be candidate genes to analyze along with deletion in *ABL-BCR* on derivative 9 (21). Therefore, an array of adjacent genes at the breakpoint and fusion regions needs to be analyzed using Bacterial Artificial Chromosome (BAC) FISH clones. We are currently working on the BACs for different chromosome 9 and 22 breakpoint and fusion regions in cases with deletion in derivative 9.

In conclusion, variant D-FISH signal pattern involving deletion in *ABL*, *BCR* or *ABL-BCR* on derivative 9 did not correlate with response to therapy. Though this is the first small study from India, the results should be validated on large scale. Tumor related genes adjacent to breakpoint and fusion region of *BCR* and *ABL* need to be analyzed using bacterial artificial chromosome (BAC) FISH for the deletion status and must be correlated along with the derivative 9 deletion and clinical response to the therapy. Our group has been developing home-brew FISH probes using BAC clones for breakpoint and fusion region of chromosome 9 and 22 for wider research application.

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Conflict of interest

We declare no conflicts of interest.

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Penta d.o.o. • Danijela Ćurčić / Frano Falkoni
A. Hebranga 20, 10000 Zagreb, Hrvatska
tel: 01 4553 290, 462 86 17 • fax: 01 4553 284
e-mail: danijela@penta-zagreb.hr • www.penta-zagreb.hr