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Characterization of Genomic Events Other than Ph and Evaluation of Prognostic Influence on Imatinib in Chronic Myeloid Leukemia (CML): A Study on 1449 Patients from India

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Abstract

Background: Analysis of Philadelphia (Ph) chromosome, a hallmark of chronic myeloid leukemia (CML) plays an important role in disease monitoring of the targeted drug Imatinib. Apart from Ph, genomic imbalances such as additional chromosomal abnormalities (ACAs) of major route occur during transformation of the disease and show negative impact on prognosis. Objective: The present study was carried out to investigate frequencies of ACAs, genomic deletions, complex Ph variants and their prognostic influences in a large cohort of newly diagnosed CML-CP (chronic phase) and CML-AP/BP (accelerated/blast phase). Material & Methods: Retrospective, single institutional study on 1367 cases of CML-CP and 82 cases of CML-AP/BP between 2009 and 2015, using conventional cytogenetics along with fluorescence *in situ* hybridization. Results: Of the 1367 patients in CML-CP, 1041 patients who completed 12 - 18 months of Imatinib therapy showed complete cytogenetic remission (CCyR) rates of 76% and 82% at 12 and 18 months respectively. Imatinib induced 81% and 33% CCyR in CML-AP and CML-BP respectively. Frequencies of ACAs in CML-CP, AP and BP were 2%, 27% and 67% respectively. Patients in chronic and AP/BP phase with ACAs showed resistance to Imatinib ($p < 0.0005$). The incidence of genomic deletions and complex Ph variants was 21% and 6.3% respectively with no comparable difference of cytogenetic response to Imatinib ($p < 0.732$ and $p < 0.210$ respectively). In a cohort of 112 patients in CCyR,

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development of new clonal abnormalities, more frequently trisomy 8 was detected in Ph negative clone. Conclusion: Our data demonstrated that Imatinib as a frontline therapy had significantly improved management of CML. However, ACAs play an important role in resistance to Imatinib, both in chronic and acute phase, which may limit sole ABL targeted therapy.

Keywords

CML, ACAs, CCyR, Genomic Deletions, Imatinib

1. Introduction

Chronic myeloid leukemia (CML) is a pleuripotent stem cell neoplasm that occurs with an incidence of 0.8 - 2.2 per 100,000 in adult men and 0.6 - 1.6 per 100,000 in adult women [1]. Incidence of CML is lower in India (5% - 8%) as compared to incidence of all leukemias in western population (14% - 20%) [1]-[3]. Philadelphia (Ph) chromosome is a hallmark of CML characterized by translocation between chromosomes 9 and 22, which results in fusion of 5'*ABL*1 on derivative 9 and 3'*BCR* on derivative 22 [4] [5]. The chimeric *BCR-ABL*1 fusion protein is a constitutively activated tyrosine kinase (TK) that leads to autophosphorylation and promotes proliferation through downstream pathways such as RAS, RAF, JUN kinase, MYC, STAT and nuclear factor-*κ*B [6] [7]. Imatinib Mesylate, a tyrosine kinase inhibitor (TKI) was found to be an efficient and potent targeted drug with complete cytogenetic remission (CCyR) in >80% of cases and 10 years of survival in 80% - 90% of cases in CML-chronic phase [8] [9].

Apart from the standard t(9;22), 3% - 8% of CML cases show either a complex Ph variant or a masked Ph [10] [11]. Acquisition of additional chromosomal abnormalities (ACAs) is one of the important features of genomic imbalances which occur during transformation of the disease from chronic phase to accelerated/blast phase in CML [12]-[15]. Prognostic significance of ACAs, more frequently major route abnormalities like Ph duplication, trisomy 8, trisomy 19, and iso chromosome 17, [i(17q)] in accelerated and blast phase have been recognized due to their association with resistance and failure of TKI [13] [15] [16]. Recent studies have shown that ACAs of major route also occur at lower rates (3% - 10%) in CML-chronic phase and their presence in diagnosis is a "warning feature" of either resistance to Imatinib or progression to blast crisis [17]-[21]. To the best of our knowledge, there are no published studies on clinical significance of ACAs, genomic deletions and complex Ph variants from India. According to international guidelines, conventional cytogenetics along with fluorescence *in situ* hybridization (FISH) are gold standard tools for identification of t(9;22), evaluation of additional chromosomal abnormalities apart from Ph, and confirmation of *BCR-ABL*1 fusion in CML cases with variant, masked Ph, cryptic insertion of *BCR-ABL*1 and genomic deletions [21]-[25]. FISH is also used as an alternative tool for an assessment of response to Imatinib and course of disease in CML cases with inadequacies of bone marrow aspirate.

The present large scale study was undertaken to analyze: 1) cytogenetic response of Imatinib as a first line therapy in newly diagnosed CML-CP and CML-AP/BP cases; 2) impact of baseline additional chromosomal abnormalities on the response rates to Imatinib in CML-CP and CML-AP/BP cases; and 3) evaluation of genomic deletions, complex variants and their prognostic impact in newly diagnosed CML-CP patients.

2. Material and Methods

Patients were diagnosed at the Department of Medical Oncology and cytogenetic studies were carried out in Cancer Cytogenetics Department, Tata Memorial Hospital, Mumbai, India between January, 2009-November, 2015. A cohort of 1367 newly diagnosed and untreated CML-CP patients consisting of 973 males and 394 females (M/F ratio of 2.5) in the age range of 16 - 82 years and 82 *de novo* cases of CML-AP/BP (AP: 52, BP: 30) consisting of 54 males and 28 females (M/F ratio of 1.9) in the age range of 15 - 65 years were retrospectively analyzed for incidence of ACAs, genomic deletions and complex variants. Diagnosis of CML-CP, CML-AP, CML-BP was based on clinical features, bone marrow morphology, immunphenotypic features and was confirmed by cytogenetic analysis [26].

Patients in chronic phase were treated with standard daily dose of 400 mg of Imatinib Mesylate. Patients in accelerated and blast phase were treated with daily dose of 600 - 800 mg of Imatinib as per recommended crite-

ria. Of the 1367 newly diagnosed and untreated CML-CP patients, 1041 patients consisting of 737 males and 304 females (M/F ratio of 2.4) in the age range of 16 - 82 years (median age 39 yrs) and 64 out of 82 cases of CML-AP/BP consisting of 42 males and 22 females (M/F ratio of 1.9) in the age range of 15 - 65 years (median age 37 yrs) were enrolled in the present study for evaluation of treatment response at 12 and 18 months. The remaining 326 patients from the CML-CP group and 18 patients from the CML-AP/BP group were not included in the study as they did not complete the 12 months of therapy at the time of analysis. Hematologic and cytogenetic responses were assessed as per updated ELN and NCCN guidelines [26]. Cytogenetic responses in CML-CP and CML-AP/BP were assessed at 3, 6, 12 and/or 18 months of Imatinib treatment.

In 2015, a cohort of 112 patients in CML-CP with complete cytogenetic remission was studied by conventional karyotyping to evaluate the emergence of new clonal abnormalities.

Cytogenetic responses were evaluated by conventional cytogenetics based on percentage of Ph positive metaphase cells. Cytogenetic response was defined as complete cytogenetic response (CCyR: No Ph positive metaphase cell), Partial cytogenetic response (PCyR: 1% - 35% Ph positive metaphase cells), Minor cytogenetic response (mCyR: 36% - 65% Ph positive metaphase cells), Minimal cytogenetic response (minCyR: 66% - 95% Ph positive metaphase cells) and No cytogenetic response (NCyR: >95% Ph positive metaphase cells).

Conventional cytogenetic studies were carried out on cultured bone marrow aspirates using standard protocol. At least 20 GTG-banded metaphase cells were analyzed at diagnosis as well as during follow-up. Additionally, FISH studies were performed in bone marrow and/or peripheral blood in both metaphase and interphase cells by using panel of probes: LSI dual colour, dual fusion *BCR/ABL*1 probe, CEP 8, LSI 19p13/19q13, LSI 21q22 and LSI *TP*53/CEP17 probes (Abbott Molecular, Delkenheim, Germany) as per manufacturer's protocol. A total of 200 interphase and 5 - 10 metaphase cells were analyzed for identification of standard *BCR-ABL*1, genomic deletions, cryptic, complex variant Ph and additional chromosomal abnormalities, particularly major route aberrations which included Ph duplication, trisomy 8, trisomy 19, trisomy 21, i(17)(q10) and Abn(17). The cut-off threshold for dual fusion *BCR-ABL*1 probe, trisomy 8, trisomy 19 and *TP*53 deletion were 2% and 5% respectively. In rare instances such as bone marrow culture failure and poor mitotic index, FISH was applied in peripheral blood for disease monitoring by using *BCR-ABL*1 signal pattern at diagnosis as a reference signal pattern for subsequent follow-up studies. A baseline threshold of 5% *BCR-ABL*1 positive interphase cells was established by comparing results of optimal and suboptimal responses of conventional cytogenetics with FISH results in substantial number of cases.

Clinical significance of additional chromosomal abnormalities, complex variant Ph and genomic deletions was evaluated by Pearson's chi-square test (SPSS version 20).

3. Results

3.1. Genomic Deletions, Complex Ph Variants and Additional Chromosomal Abnormalities in CML-CP

Of the total 1367 patients with CML-CP, 995 patients (73%) showed standard Ph: t(9;22) with typical dual *BCR-ABL*1 fusion signal pattern (1R1G2F), whereas complex variant Ph: t(v;9;22) with 2R2G1F signal was identified in 86 (6.3%) cases (**Figure 1(a)** and **Figure 1(b)**). Frequency of atypical *BCR-ABL*1 with genomic deletions was 21% (286 cases) which included 12% (165 cases) with der(9q) deletion, 5.3% (73 cases) with 5'*ABL*1 deletion, 3% (43 cases) with 3'*BCR* deletion and 0.4% (5 cases) with der(22q) deletion (**Figure 1(c)-(f)**). The frequencies of standard t(9;22), complex variant Ph and genomic deletions were in the similar range in a cohort of 1041 out of total 1367 CML-CP cases, who were evaluated for Imatinib response (**Table 1**). Ph duplication was detected in 21 (2%) cases of CML-CP, of which 18 (1.7%) cases displayed Ph duplication at diagnosis and 3 (0.3%) cases developed ACAs during the course of disease.

3.2. Cytogenetic Response to Imatinib Mesylate in CML-CP

Of the total 1041 patients evaluated for Imatinib response, 980 (95%) patients achieved complete hematological response, 796 (76%) patients achieved complete cytogenetic response (CCyR), 146 (14%) patients had partial cytogenetic response (PCyR) and 99 (10%) patients displayed minor/minimal/no cytogenetic response (mCyR/

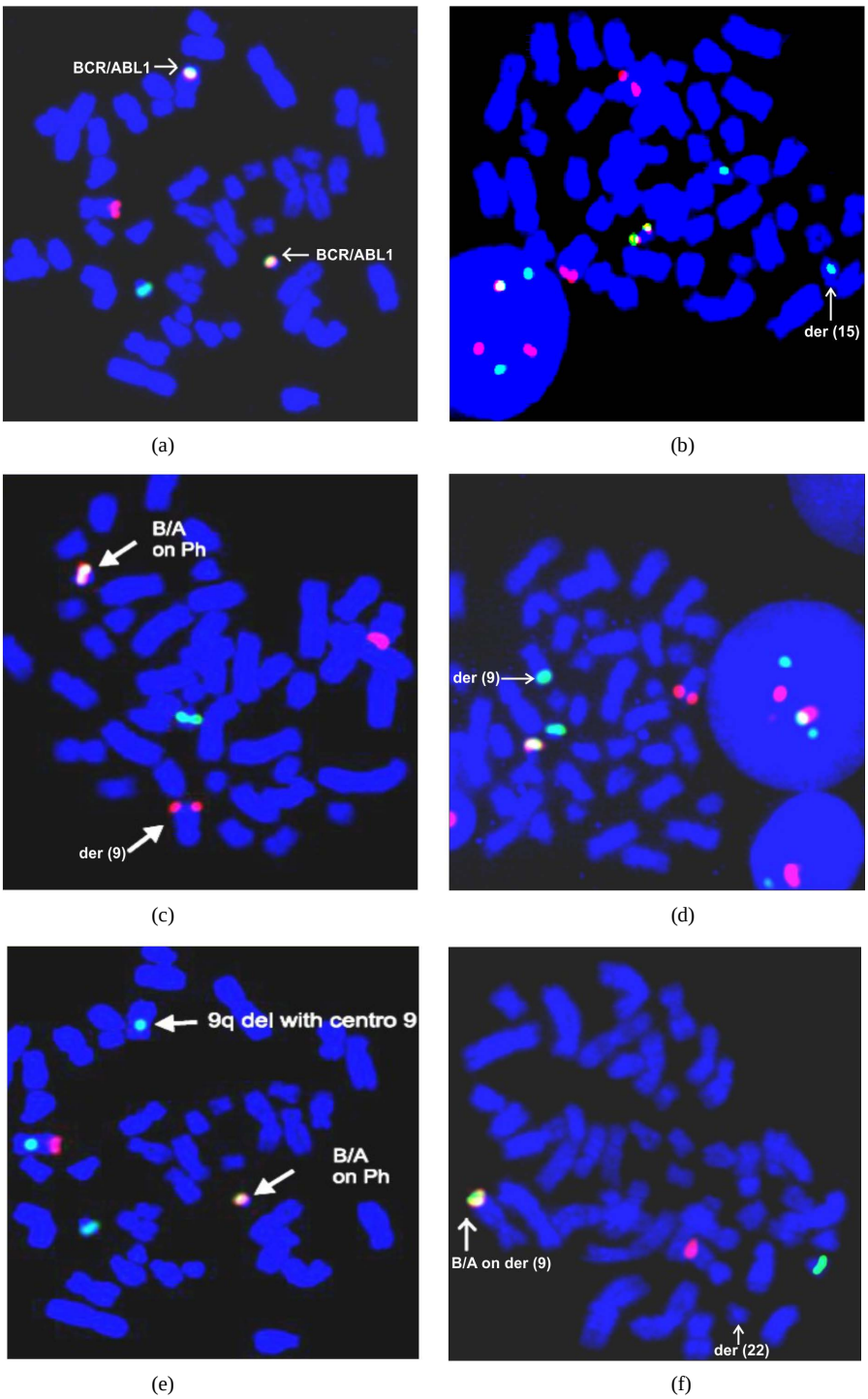


Figure 1. (a) Dual fusion *BCR-ABL*1 probe on metaphase cell shows standard *BCR/ABL*1 dual fusion (1R1G2F). (b) Dual fusion *BCR-ABL*1 probe on metaphase and interphase cell shows complex Ph variant with *BCR/ABL*1 fusion t(9;22;15)(q34;q11.2;q11)(2R2G1F). (c) Dual fusion *BCR-ABL*1 probe on metaphase cells shows atypical *BCR-ABL*1 on der(22) with 3'*BCR* deletion (2R1G1F). (d) Dual fusion *BCR-ABL*1 probe on metaphase and interphase cell shows atypical *BCR/ABL*1 on der(22) with 5'*ABL*1 deletion (1R2G1F). (e) Dual fusion *BCR/ABL*1 probe on metaphase cell shows atypical *BCR/ABL*1 on der(22) with 9q deletion on der(9)(1R1G1F). (f) Dual fusion *BCR/ABL*1 probe on metaphase cell shows atypical *BCR/ABL*1 on der(9) with 22q deletion on der(22)(1R1G1F).

minCyR/NCyR) after 12 months of Imatinib therapy (Figure 2). Further follow-up at 18 months showed CCyR in 82% and PCyR in 8% cases (Figure 2).

3.3. Cytogenetic Response of CML-CP with Genomic Deletions, Complex Ph Variant and Additional Chromosomal Abnormalities

Cytogenetic response varied in CML-CP patients with Ph duplication as well as in those without Ph duplication at diagnosis ($p < 0.0005$) (Table 2). Patients with Ph duplication had 30% optimal response (CCyR + PCyR). Patients with development of Ph duplication as a process of clonal evolution had suboptimal response to Imatinib (two with minCyR and one with NCyR).

CML-CP patients with genomic deletions and those with complex Ph variant excluding patients with ACAs did not show comparable differences of cytogenetic response to Imatinib ($p < 0.732$ and $p < 0.210$ respectively) (Table 2).

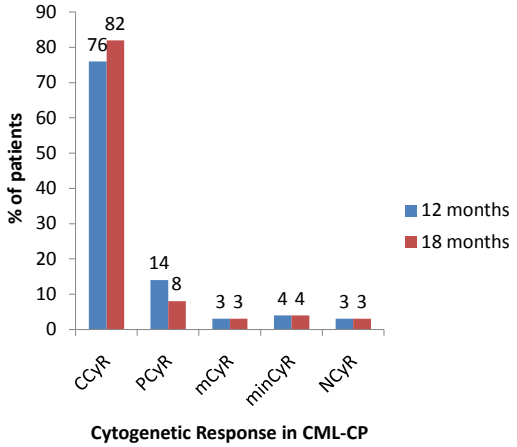


Figure 2. Cytogenetic response to Imatinib at 12 Months & 18 Months in CML-CP(CML-Chronic phase). CCyR: Complete cytogenetic response, PCyR: Partial cytogenetic response, mCyR: Minor cytogenetic response, minCyR: Minimal cytogenetic response. NCyR: No cytogenetic response.

Table 1. Frequency of Genomic deletions and complex variants in CML-CP (n = 1041 cases).

Typical *BCR-ABL1*	Atypical *BCR-ABL1*				Complex Ph Variant t(v;9;22)
	9q deletion	5'*ABL1* deletion	3' *BCR* deletion	22q deletion	
1R1G2F	1R1G1F	1R2G1F	2R1G1F	1R1G1F	2R2G1F
767	117	55	30	4	68
73.6%	11.3%	5.3%	3%	0.4%	6.5%

Table 2. Cytogenetic response in CML-CP (CML-Chronic Phase) patients with additional chromosomal abnormalities (ACAs), genomic deletions and complex Ph variants (n = 1041 cases).

	CCyR + PCyR	mCyR + minCyR + NCyR	*p*-value
With ACAs	4 (0.4%)	14 (1.3%)	0.0005
Without ACAs	936 (90%)	87 (8.4%)	
With Genomic deletion	189 (18.5%)	16 (1.6%)	0.732
Without Genomic deletion	749 (73%)	70 (6.8%)	
With Complex variants	65 (6.4%)	3 (0.3%)	0.210
Without complex variants	872 (85%)	84 (8.2%)	

CCyR: Complete cytogenetic response, PCyR: Partial cytogenetic response, mCyR: Minor cytogenetic response, minCyR: Minimal cytogenetic response, NCyR: No cytogenetic response.

3.4. Additional Chromosomal Abnormalities (ACAs) in CML-AP/BP and Cytogenetic Response to Imatinib Mesylate

Of the 82 patients in CML-AP/BP, 64 patients who had completed 12 months of therapy, complete hematological response was 90% in CML-AP and 70% in CML-BP. Overall complete and partial cytogenetic responses in 43 patients with CML-AP were 81% and 9% respectively. On the other hand, CCyR and PCyR in 21 patients in blast phase were 33% and 5% respectively (**Figure 3**). In a cohort of 82 patients with CML-AP/BP, frequency of ACAs was 41% (34/82 cases), of which 27% were in AP and 67% were in BP. The ACAs included major route abnormalities such as Ph duplication (23%), trisomy 8 (17%), trisomy 19 (10%), trisomy 21 (7%), 17p deletion/-17 (2.4%) and del(7q) (1.2%). Incidence of more than one ACAs was higher in CML-BP (6/30 cases: 20%) than CML-AP (4/52 cases: 8%) (**Figures 4(a)-(c)**; **Table 3**). One of the BP patients with masked Ph had duplication of der(9) with *BCR-ABL*1 with concurrent loss of normal 9 (**Figure 5**). Cytogenetic response to Imatinib in AP/BP patients with major route ACAs was significantly poor as compared with those with no ACAs ($p < 0.0005$) (**Table 4**).

One case of CML-CP, a 25 year old female, was diagnosed with 98% cells with *BCR-ABL*1 in February, 2014. After receiving 400 mg of Imatinib for 6 months, she showed suboptimal response with 60% *BCR-ABL*1 positive cells and evolved to promyelocytic blast crisis two months later in October, 2014. FISH analysis revealed 90% *BCR-ABL*1 and 98% *PML-RARA* (**Figure 6(a)** and **Figure 6(b)**). The patient was treated with arsenic trioxide and died of CNS bleeding with bilateral pneumonia and sepsis within 10 days of initiation of treatment.

Table 3. Frequency of additional aberrations in CML-AP (Accelerated Phase) (14/52: 27%) and CML-BP (Blast Phase) (20/30: 67%) cases (n = 82).

Chromosomal abnormality	No of cases (%)
Duplication Ph	19 (23%)
Trisomy 8	14 (17%)
Trisomy 19	8 (10%)
Trisomy 21	6 (7%)
Abn (17)	2 (2.4%)
del(7q)	1 (1.2%)

Table 4. Cytogenetic response in CML-AP and CML-BP cases with and without additional cytogenetic abnormalities (ACAs) (n = 64).

	CCyR + PCyR	mCyR + minCyR + NCyR	p-value
With ACAs	11 (42.3%)	15 (58%)	0.0005
Without ACAs	36 (95%)	2 (5%)	

CCyR: Complete cytogenetic response, PCyR: Partial cytogenetic response, mCyR: Minor cytogenetic response, minCyR: Minimal cytogenetic response, NCyR: No cytogenetic response.

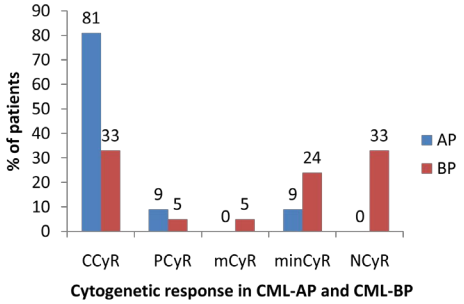


Figure 3. Cytogenetic response to Imatinib at 12 Months in CML-AP (CML-Accelerated) and CML-BP (Chronic-Blast phase). CCyR: Complete cytogenetic response, PCyR: Partial cytogenetic response, mCyR: Minor cytogenetic response, minCyR: Minimal cytogenetic response. NCyR: No cytogenetic response.

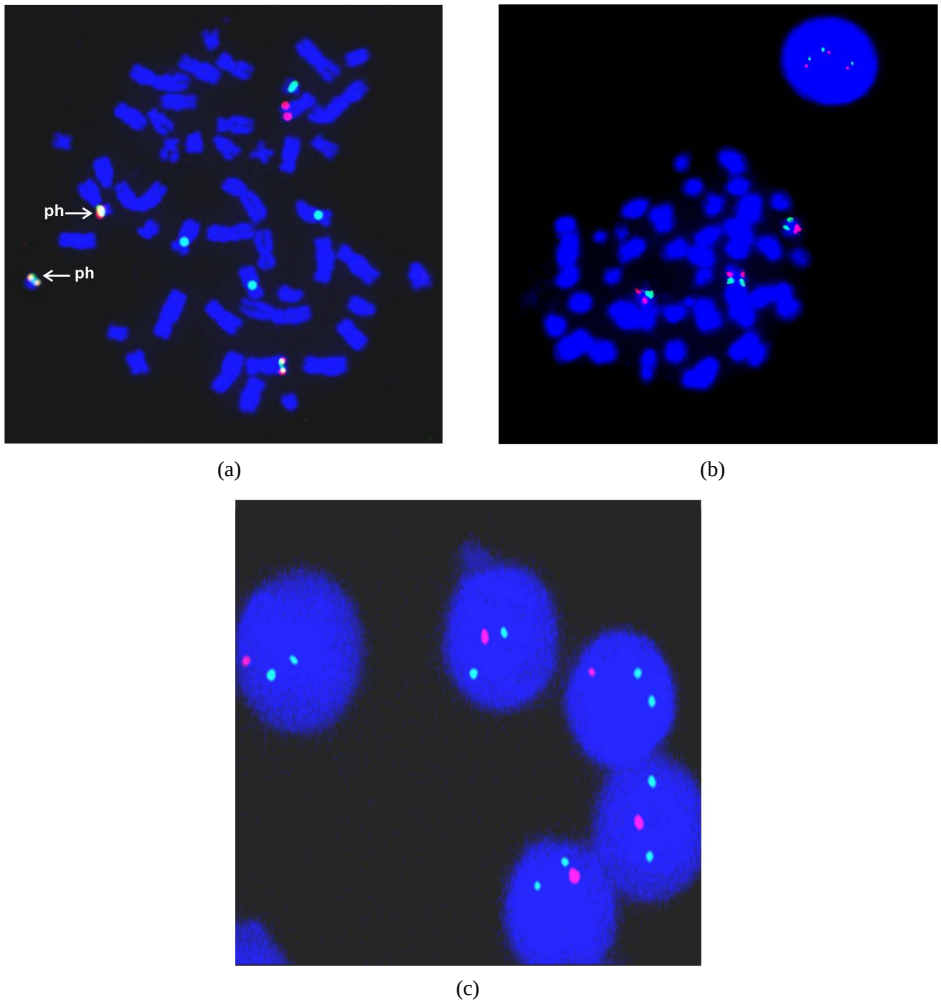


Figure 4. (a) Metaphase-FISH with combination of *BCR/ABL*1 probe and CEP 8 shows duplication of Ph with *BCR/ABL*1 (1R1G3F) and trisomy 8 (Aqua colour signals on chromosome 8). (b) LSI 19p13/19q13 probe on metaphase and interphase cell shows trisomy 19. (c) LSI 17(p13.1)(*TP*53)/CEP17 probe on interphase cells shows allelic loss of *TP*53 (1R2G).

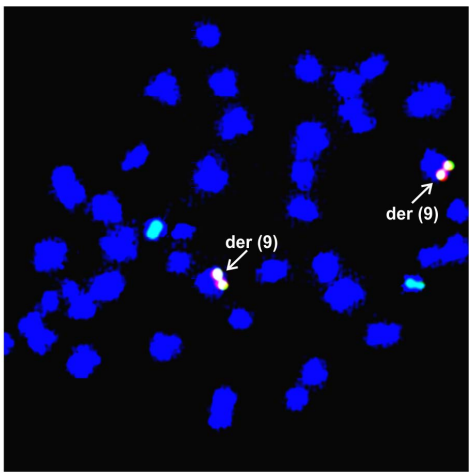
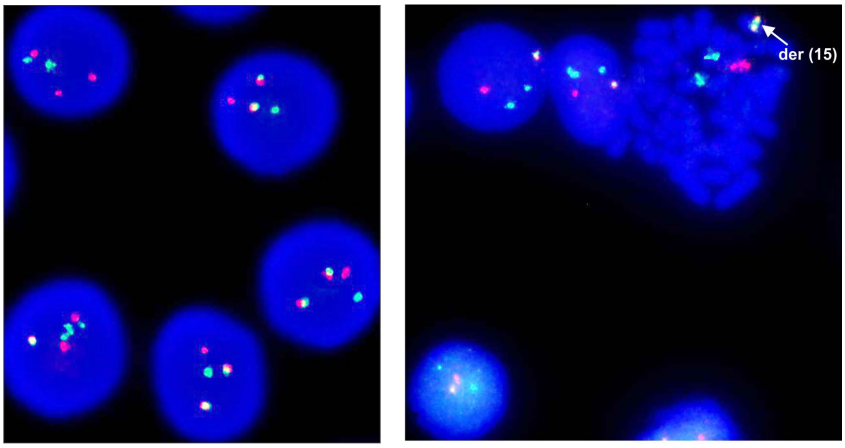


Figure 5. Dual fusion *BCR/ABL*1 probe on metaphase cells shows masked Ph with duplication of der(9) with *BCR/ABL*1 and loss of normal 9.



(a) (b)

Figure 6. (a) Dual fusion *BCR/ABL*1 probe on interphase cell shows standard *BCR/ABL*1 dual fusion (1R1G2F); (b) Dual fusion *PML/RARA* probe on interphase cell shows atypical *PML/RARA* on der(15) with residual *PML* deletion (1R2G1F).

3.5. Development of New Clonal Abnormalities in Ph Negative Clone after Imatinib Treatment in CML-CP

Among the 112 patients in CML-CP with complete cytogenetic remission, four males (3.8%) in the age range of 18 - 60 yrs achieved CCyR at 4 months-2 yrs and developed new clones of trisomy 8 (3 cases) and trisomy 11 (1 case) either at the time of induction of CCyR or 15 - 24 months later after attaining CCyR. The clone size was 25% - 55%. None of these patients showed myelodysplasia-related changes in the marrow (**Figure 7**).

4. Discussion

Apart from conventional cytogenetics, parallel interphase- and metaphase-FISH application could efficiently detect cryptic *BCR-ABL*1 and *BCR-ABL*1 with genomic deletions; help in identification and confirmation of complex Ph variant or masked Ph as a result of 3-way and 4-way interchromosomal translocation apart from 9 and 22.

FISH with cocktail of probes was found to be the best strategy to identify ACAs apart from *BCR-ABL*1. The establishment of base-line by comparing I-FISH results with percentage of t(9;22) by conventional cytogenetics in bone marrow cultures at various phases of the disease helped disease monitoring in peripheral blood specimens. Several labs have adopted this strategy in peripheral blood specimen in case of suboptimal bone marrow [24] [25]. Metaphase-FISH helped right interpretation of signal pattern of interphase cells which helped in the identification and confirmation of abnormalities like 1R1G1F which can be a result of *BCR-ABL*1 with der(9q) deletion or der(22q) deletion. A rare case of CML-BP from our study showed interphase signal pattern of 2G2F with dual fusion of *BCR-ABL*1 which turned out to be duplication of derivative 9 with *BCR-ABL*1 fusion on both der(9) along with concurrent loss of normal 9 homologue in the process of blastic phase. A similar finding has been reported earlier from our centre [22].

The peak onset of CML at younger age between 30 - 40 years in Indian population is evident from present large scale study and studies from other centers across India [3]. Survey of complete cytogenetic response rates of 40% - 80% in previous reports from our center and review of CML conference indicates a trend towards superior response and efficacy of Imatinib Mesylate [3] [27]. The variable rates of CCyR in previous studies could be due to use of Interferon-α and/or other chemotherapy regimens preceding Imatinib. Treatment of Imatinib as a front line therapy in early phase of CML-CP with systematic follow up showed improved CCyR as is evident in present studies with 80% CCyR in CML-CP [8] [9] [19] [28].

In the present study, analysis of ACAs in CML-CP was restricted only to Ph duplication. Despite the potency of frontline Imatinib, suboptimal response to patients with Ph duplication confirmed the importance of Ph duplication as “warning feature”. Moreover, these findings indicate that careful monitoring of sole Ph duplication at diagnosis is of utmost importance in CML-CP [17] [18] [20] [21] [26].

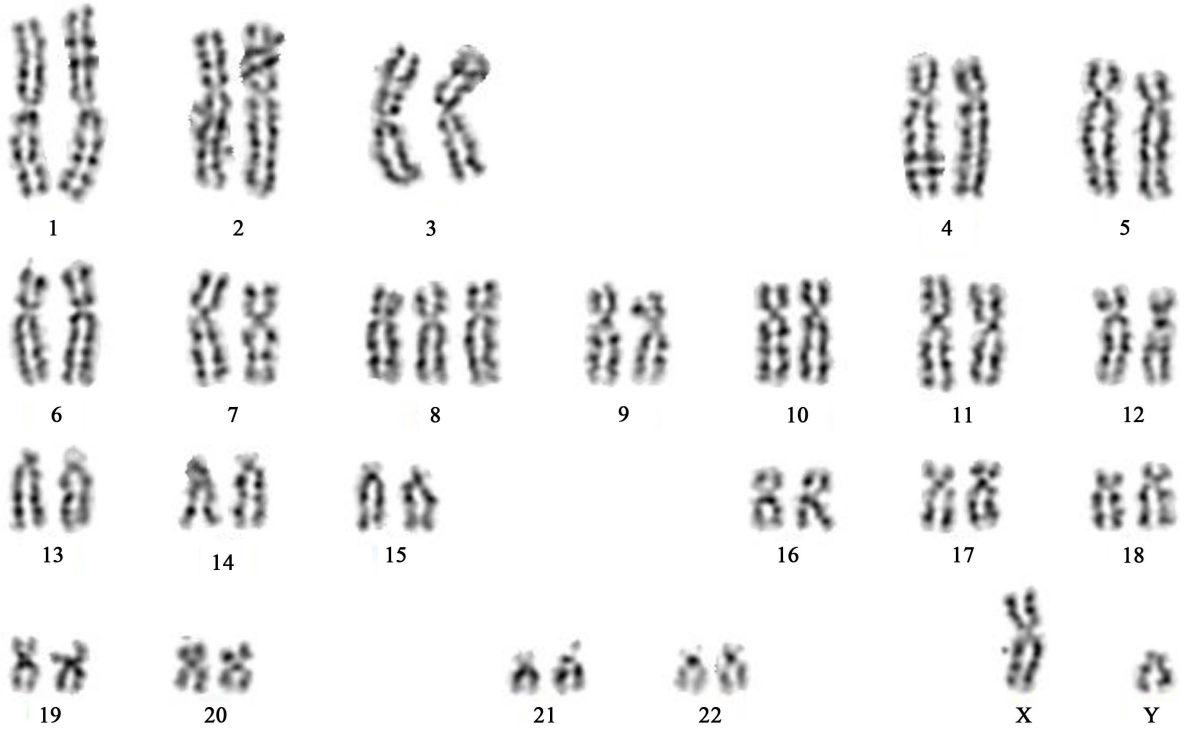


Figure 7. Ph negative karyotype showing trisomy 8.

Genomic deletions adjacent to *BCR* and *ABL*1 breakpoints occur in 15% - 30% of CML patients as was observed in our population (21%) [17] [29]. Although previous studies from other countries have shown poor prognostic impact of genomic deletions in CML-CP patients who had received Interferon-α and conventional chemotherapy before the Imatinib era [30] [31], our studies provided added evidence that genomic deletions do not influence the response to Imatinib therapy in terms of hematological and cytogenetic response [29] [32]. Frequency of complex Ph variant as a result of involvement of 3rd/4th chromosome including masked Ph was 6.3% in the present study which is in concordance with reported studies [11]. Our findings of negative impact of complex Ph variant to Imatinib are consistent with literature [11] [33].

As evident from other studies, patients in blast phase had suboptimal and/or poor response to Imatinib [15] [16] [34]. Comparatively, better response to patients in *de novo* accelerated phase in our cohort is supported by a recent study by Ohanian *et al* [35]. In the present study, we focused on the analysis of major route abnormalities viz., Ph duplication, trisomy 8, trisomy 19 and 21, abnormalities of 17 and chromosome 7. As reported previously, CML-BP in our cohort had higher incidence of ACAs (67%) than CML-AP (27%) [12] [16] [19] [36]. The development of additional chromosomal abnormalities with other mutational events shows strong evidence of increased genomic instability with progression of disease. Therapeutic influence of alkylating agents such as Busulfan was suspected on the development of ACAs in evolution to blast phase before Imatinib era [37]. We had previously reported higher prevalence of ACAs in BP patients who had received Busulfan/Hydroxyurea (70% vs 44%) in chronic phase [12]. The presence of ACAs in the present cohort of *de novo* CML-AP/BP patients indicates inherent genomic instability which may be further aggravated by cascade of genomic events in the process of transformation [38].

In consistent with literature, our findings regarding the cytogenetic response in CML-BP indicated that patients with additional chromosomal abnormalities are associated with poor response to TKI [15] [16] [21] [26] [34] [36]. Although global results in different models give better hopes for new 2nd and 3rd generation TKI in CML-BP, genetic heterogeneity may limit sole ABL targeted therapy. Allogenic stem cell transplantation seems to be an important option with careful, sensitive monitoring of disease based on genomic markers responsible for disease progression.

As per recommended guidelines of disease monitoring, clonal hematopoiesis with development of MDS-related abnormalities especially monosomy 7 in Ph negative clone is a “warning feature”. There are reports of

emergence of new clonal abnormalities in Ph negative clone with incidence of 3% - 10% in CML patients treated with Imatinib Mesylate [18] [39] [40]. As observed in earlier reports and in the present study, the emergence of new clone(s) occur either at the time of induction of CCyR or 2 - 3 yrs later after achievement of CCyR. Our study indicates that trisomy 8 is common in Ph negative clone in CML in CCyR achieved by Imatinib and that has no impact on clinical outcome [18] [36] [40]. The mechanism of the emergence of new clones in Ph negative clone in remission after Imatinib is not known, however unknown late side effects of TK inhibitor in the form of DNA damage and repair cannot be denied. However, long term systematic monitoring of these patients by cytogenetics is advisable.

5. Conclusion

Our data demonstrated that Imatinib as a frontline therapy had significantly improved management of CML in terms of cytogenetic response as per International guidelines. Our data confirmed that additional chromosomal abnormalities of major route were key factors which played an important role in resistance or failure to Imatinib not only in CML-BP but also constituted a "warning feature" in CML-CP. In agreement with recent observations, although escalation of Imatinib/2nd generation TKI comparatively showed better response, more intensive therapy is needed for patients with ACAs. In addition to major route additional chromosomal abnormalities, screening of the acquired kinase mutations is necessary and may be useful for selection of therapeutic options such as stem cell transplantation or TKI in combination with other chemotherapeutic regimens. The application of conventional cytogenetics and/or FISH with a cocktail of probes has remained a gold standard and is mandatory at diagnosis and during the course of the disease for the right interpretation of the cytogenetic picture and close monitoring of the disease which may help early prediction of therapeutic response/progression of the disease.

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