

Original Article

## Investigating Breakpoint Cluster Region-Abelson 1(BCR-ABL) (BCR-ABL1) signal patterns across chronic myeloid leukemia morphologic phases and its impact on molecular response

Udaya Sundarajan,<sup>MD</sup><sup>1</sup>, Karthik Dhandapani,<sup>DM</sup><sup>2</sup>, Prerna Walia,<sup>MD</sup><sup>2</sup>, Saurabh Jyoti Goswami,<sup>MD</sup><sup>1</sup>, Prajitha P,<sup>MD</sup><sup>2</sup>, Biren Parikh<sup>ID</sup>,<sup>MD</sup><sup>2</sup>, Pina Trivedi<sup>ID</sup>,<sup>PhD</sup><sup>3</sup>, Dharmesh M Patel,<sup>PhD</sup><sup>3</sup>

Departments of <sup>1</sup>Pathology, <sup>2</sup>Oncopathology, <sup>3</sup>Cytogenetics, Gujarat Cancer and Research Institute, Ahmedabad, Gujarat, India.

### ABSTRACT

**Objectives:** For the diagnosis of chronic myeloid leukemia (CML) FISH test confirms the presence of BCR-ABL translocation in CML. It also shows whether the signal pattern is typical or atypical. CML has to be monitored through quantitative real time polymerase chain reaction(qRT-PCR) for Breakpoint Cluster Region-Abelson 1(BCR-ABL1) transcripts to evaluate the achievement of major molecular response (MMR). However, the significance of the association of atypical signal patterns with major molecular response (MMR) achievement remains to be studied.

**Material and Methods:** In all newly diagnosed cases of CML, peripheral blood, bone marrow examination, European treatment and outcome study score(EUTOS) score assessment, FISH and real time polymerase chain reaction(RT-PCR) were performed.

**Results:** Among 369 CML cases, 70 (19%) cases of blast crisis and 299 (81%) cases of chronic phase were reported. Among these 299 cases presenting with chronic myeloid leukemia - chronic phase (CML-CP) on bone marrow biopsy, increased fibrosis was noted in 29 (9.7%) cases. On FISH, atypical patterns were present in 57.4% cases of chronic myeloid leukemia-blast crisis (CML-BC), 65.5% of CML with fibrosis, and 29.6% cases of CML-CP. RT-PCR after one year of therapy showed MMR in 62.9% of cases who had a typical signal pattern on FISH, whereas the number dropped to a mere 7.9% in patients with an atypical pattern on FISH. Independent of EUTOS risk stratification, CML-CP cases with atypical FISH patterns showed a significant difference in the attainment of MMR compared to CML-CP cases with typical patterns (9.1% vs 65.9%).

**Conclusion:** Ours is one of the few studies that analyzed and compared FISH patterns with morphological diagnosis and MMR status on RT-qPCR in such a large cohort of patients with CML CML BC and CML-CP with fibrosis cases significantly show more atypical patterns compared to CML-CP cases. Also, cases with atypical patterns attained MMR significantly in fewer numbers compared to cases with typical patterns. Hence FISH test with emphasis on atypical patterns can also be considered for the prognostication of patients with CML.

**Keywords:** BCR-ABL, CML, q-PCR, variant FISH patterns, prognosis

### INTRODUCTION

Chronic myeloid leukemia (CML) is a genetically driven myeloproliferative neoplasm characterized by clonal proliferation of myeloid cells in the bone marrow and their

uncontrolled accumulation in the blood. It is consistently associated with Breakpoint cluster region - Abelson murine leukemia fusion oncogene (BCR-ABL1) fusion gene located

\*Corresponding author: Dr. Biren Parikh, Department of Oncopathology, Gujarat Cancer and Research Institute, Civil campus, Ahmedabad, Gujarat, 380016, India. [birenparikh2002@rediffmail.com](mailto:birenparikh2002@rediffmail.com)

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in the Philadelphia chromosome (Ph). The discovery of BCR-ABL1 has revolutionized the treatment of CML through targeted drugs that inhibit the tyrosine kinase activity of the BCR-ABL oncoprotein. Tyrosine kinase inhibitors (TKIs) are the standard therapy for CML. This is the most common leukemia encountered in India.<sup>[1]</sup> For many years, conventional karyotyping was used as the sole diagnostic tool for t(9;22). However, it can result in false negative in around 5% of all CML patients.<sup>[2]</sup> FISH (Fluorescence In Situ Hybridization) test not only confirms the presence of BCR-ABL translocation in CML, but also shows the type of FISH signal pattern. Atypical signal patterns usually represent a deletion on the translocated derivative part of either chromosome 9 and/or 22 or an additional Philadelphia chromosome. Dual color-Dual fusion BCR/ABL1 probe is a sensitive, specific, and efficient way to establish initial diagnosis of CML along with minute details like submicroscopic deletions. Also, response to treatment with TKIs should be monitored through quantitative Reverse Transcriptase- Polymerase chain Reaction (qRT-PCR) for BCR-ABL copy numbers for achievement of major molecular response(MMR). In addition, the EUTOS score should be used to risk-stratify the CML-CP patients, which predicts the achievement of MMR.<sup>[3]</sup> Thus, we monitored BCR-ABL transcripts following one year of therapy and studied their association with different FISH patterns and EUTOS risk groups.

Only very few studies are available in the literature regarding atypical FISH patterns and their association with disease progression.<sup>[4]</sup> However, none of the studies have analyzed atypical FISH pattern correlation with molecular response. This is the first Indian study that analyzed and compared typical and atypical FISH patterns with different phases of chronic myeloid leukemia.

## MATERIAL AND METHODS

Our study is a descriptive, observational study with prospective, consecutive sampling. The study was conducted over a period of 18 months at a tertiary cancer centre.

All newly diagnosed cases of CML were included. All the required clinical details of the patients were taken from the electronic case records and patients' case files. Classification of CML was performed through peripheral smear, bone marrow aspiration, and biopsy examination. Peripheral smear and bone marrow aspiration slides were stained using Wright stain. Bone marrow biopsy slides were stained using Hematoxylin and Eosin (H and E) stain. Reticulin

stain was used to classify CML-CP cases with increased fibrosis (WHO grade 2 or more) at presentation.<sup>[5]</sup> The EUTOS score was calculated based on spleen size and basophil count at the time of diagnosis in all CML-CP cases and stratified as low or high risk accordingly.<sup>[3]</sup> Also, whenever required, BM samples were processed for eight-color MFC immunophenotyping using the bulk lyse and stain method.

## Treatment regimen

All CML-CP cases were risk-stratified using the EUTOS score and were given either first-generation tyrosine kinase inhibitor (TKI) (imatinib) for low-risk or second-generation TKI (Dasatinib) for high-risk cases. For CML myeloblastic crisis, the 7+3 regimen (cytarabine daunorubicin) with imatinib, and for lymphoblastic crisis, the BFM-90 protocol was given with imatinib.

## FISH Procedure

Dual color DUAL Fusion FISH for BCR-ABL1 was performed on bone marrow aspirate samples at diagnosis. At least 200 well-visualized cells with well-delineated signals were evaluated. The type of signal pattern in FISH was reported in each case, and the presence of a fusion signal in any number of cells was considered as Philadelphia positive(t(9;22)).

## Molecular response assessment using real-time PCR

Post-treatment blood/bone marrow samples after one year were collected to evaluate for molecular response using quantitative real-time PCR. The attainment of major molecular response (MMR) was assessed based on the ratio of fusion copy numbers.

## Statistical analysis

The data was collected in a Microsoft Excel master sheet. Both Microsoft Excel and the statistical package for Social Sciences (SPSS) version 20 software were used for statistical analysis. The continuous data, like age, is expressed as a median with a range. The association and significance between discrete data have been determined using the Chi-square test.

## RESULTS

During the study period of 18 months from January 2022 to June 2023, 369 cases of CML were reported, with a median age of 41 years. The youngest age of presentation

in our study group was 9 years, while the oldest age was 80 years. The male-to-female (M:F) ratio among these cases were 2:1.

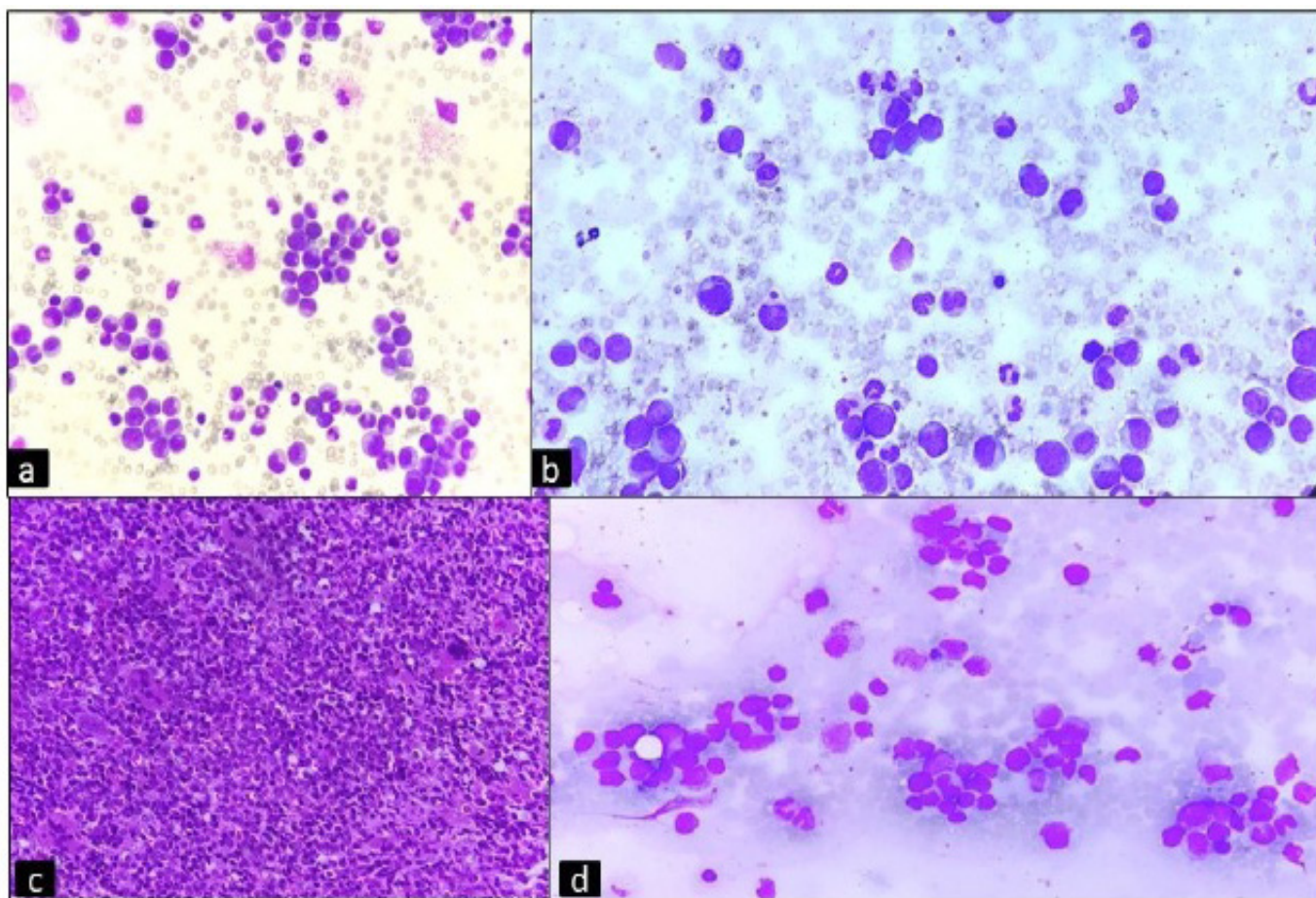
### Morphological subtyping among the cases

It revealed 70(19%) cases of blast crisis and 299 (81%) cases of chronic phase [Figures 1 and 2]. Among the 299 cases presenting with CML-CP on bone marrow biopsy, increased fibrosis was noted in 29 cases (9.7%). Out of 70 cases of blast crisis, 16 cases had extramedullary blast proliferation (22.8%) (Illustration: 1). 53 of these cases had myeloid blast crisis, while 17 cases presented with lymphoblastic crisis. Among the 16 extramedullary blast crises, lymph nodes and cerebrospinal fluid were the common sites to be involved (25% each), followed by bone, ascitic, and pleural fluid

[Figure 3]. Out of 299 cases of CML-CP, 69 cases (23.1%) were categorized as low risk and 230 cases (76.9%) as high risk based on the EUTOS scoring system.

### FISH findings in these cases

FISH was performed in all 369 cases, which revealed presence of the Philadelphia chromosome (t(9;22)) in all these cases. Also further based on the fluorescent signals, the patterns were reported as typical or atypical [Figure 4]. Out of 369 cases, 239 (64.8%) had typical FISH patterns, and remaining 130 cases (35.2%) had a typical FISH patterns [Table 1]. 10 FISH signal patterns in CML, including one typical [Figure 4a and b] and 9 atypical ones [Figure 4c-h]. The exact number of cases in each of the morphological subgroups with their specific FISH pattern is given in the table.

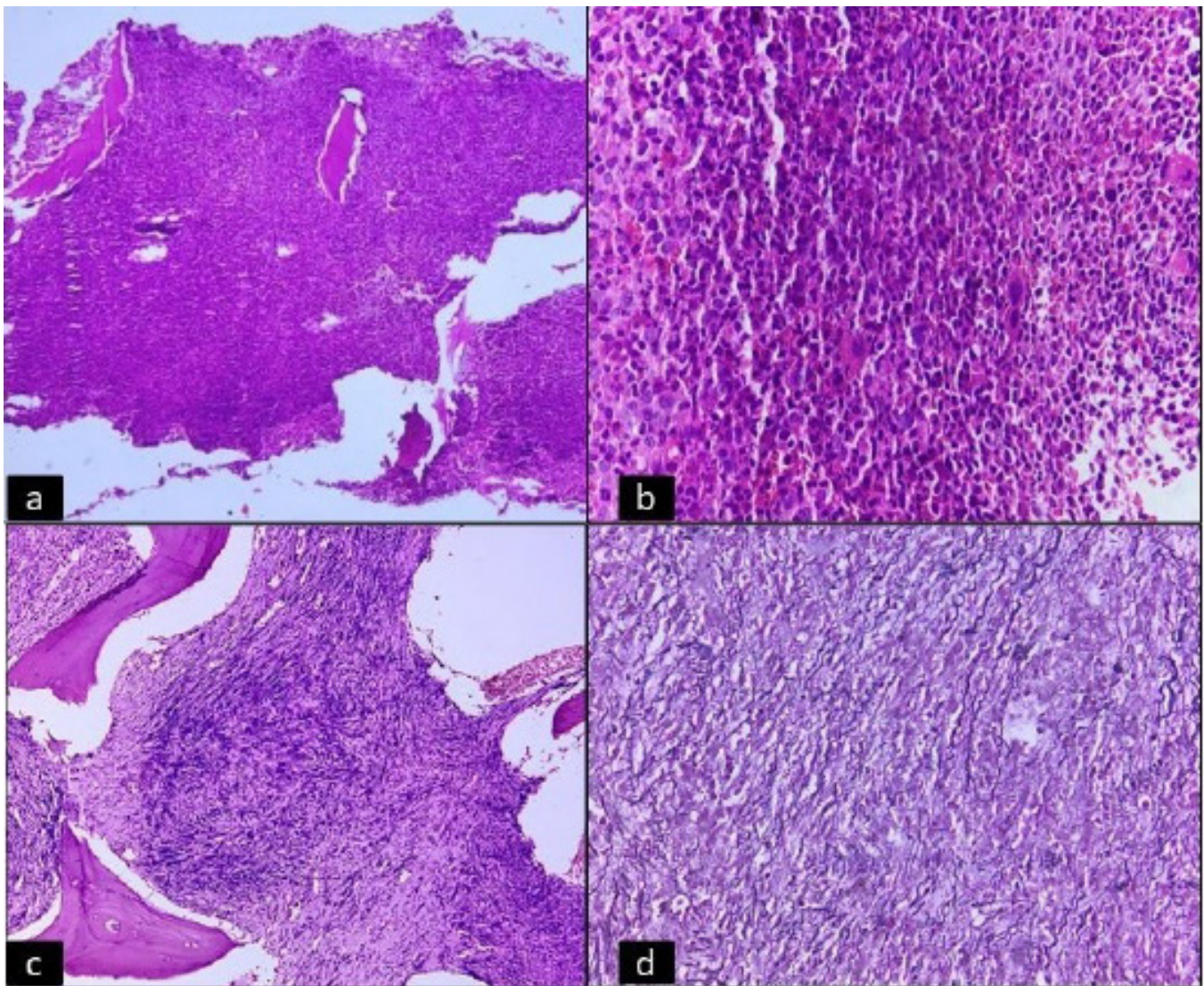


**Figure 1:** (a) Peripheral blood leucocytosis, granulocytic left shift, basophilia and eosinophilia - CML in chronic phase (20x, Wright stain). (b) and (c) Bone marrow aspirate and biopsy showing myeloid bulge with prominent myeloblasts- CML in myeloblastic crisis (b) - 40X, Wright stain. (c) - 40X, Hematoxylin and Eosin). (d) Bone marrow aspirate showing prominent blasts in the background of myeloid series proliferation- CML in Lymphoblastic crisis (40X, Wright).

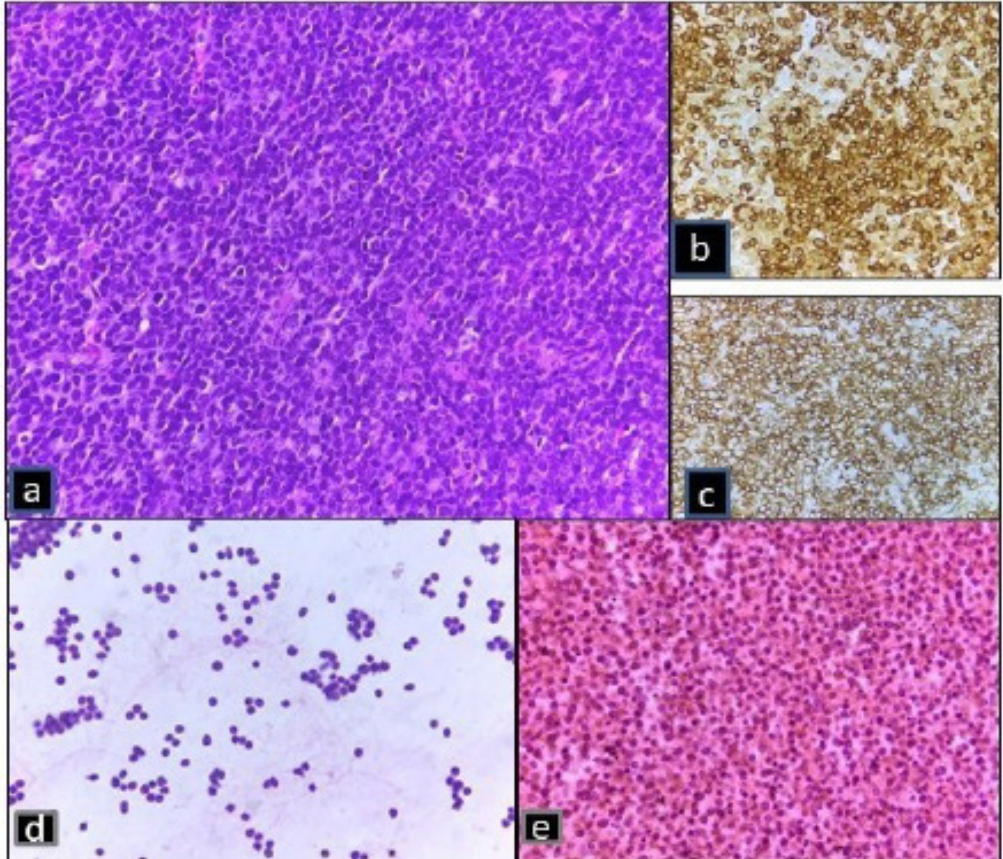
**Table 1:** FISH pattern distribution in CML cases

DIAGNOSIS	101G2F (Typical)	101 G1F	101 G1F	101 G4F	102 G1F	201 G3F	201 G1F	202 G1F	202 G2F	202 G4F	Total
CML-CP	190	32	6	0	10	1	17	13	1	0	270
CML-CP with fibrosis	10	7	5	1	2	0	2	3	0	1	29
CML-BC	33	9	0	0	5	0	3	2	0	0	54
CML-BC with extramedullary involvement	6	3	0	1	3	0	0	3	0	1	16

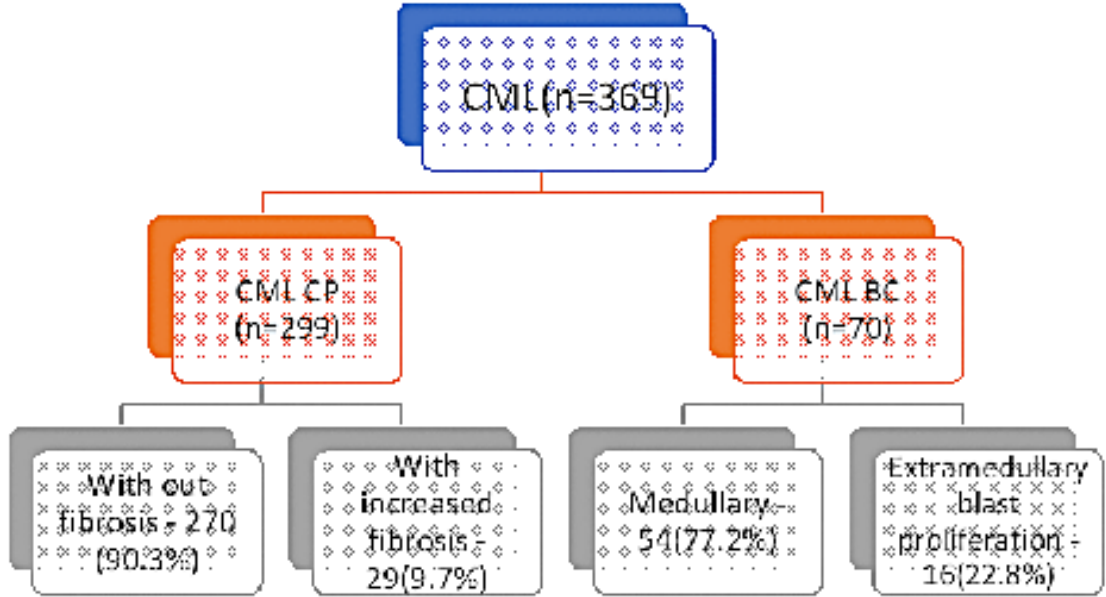
CML: chronic myeloid leukemia;- CML-CP: Chronic myeloid leukemia chronic phase; CML-BC: Chronic myeloid leukemia- Blast crisis



**Figure 2:** (a and b) CML-CP; (a) Bone marrow biopsy showing hypercellular marrow with no fat spaces in the marrow fragments (4x, H and E). (b) Granulocytic hyperplasia with all stages of maturation and scattered dwarf megakaryocytes (40x, H and E). (c) Bone marrow biopsy shows fibrosis with streaming of cells. (10x, H and E). (d) Reticulin stain in the same case demonstrated increased reticulin fibres with many intersections- Grade 2 fibrosis (40x, Reticulin). H and E: Hematoxylin and eosin



**Figure 3:** (a) Lymph node showing granulocytic sarcoma(40X, Hematoxylin and eosin). The blasts are positive for Myeloperoxidase (MPO). (b) and CD34 (c) (40X, Immunohistochemistry). (d) Cerebrospinal Fluid shows numerous myeloblasts in a clear background 40x, May-Grunwald-giemsa stain (MGG). (e) Pleural fluid cytology - highly cellular with sheets of myeloblasts – Extramedullary blast crisis (40X, Papanicolaou stain)



**Illustration 1: Morphological subtyping in CML cases**

CML: Chronic myeloid leukemia, CML CP: Chronic myeloid leukemia- chronic phase, CML BC: Chronic myeloid leukemia- Blast crisis

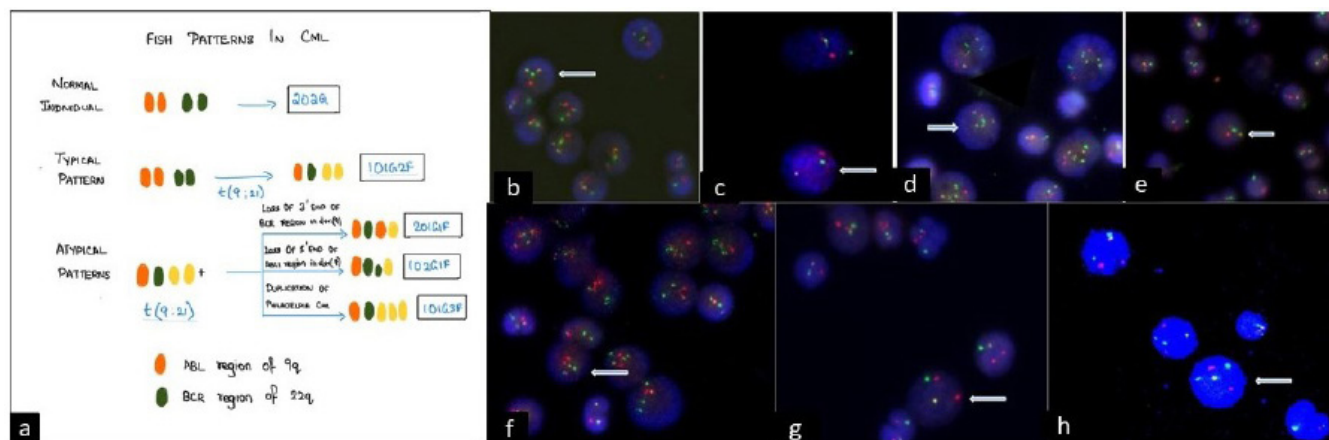


Figure 4: (a)-FISH images showing Typical and atypical patterns. (b)-101G2F (Typical), (c)- 101G1F, (d)- 101G3F, (e) - 102G1F, (f)- 201G3F , (g)- 201G1F and (h) - 202G1F (White arrow in each figure points to the classical appearance of the respective FISH pattern).

### Correlation of FISH patterns with morphology

Table 2 illustrates the distribution of typical and atypical FISH patterns among each diagnostic subgroup.

**Table 2: Typical and atypical FISH patterns in CML cases**

Sr. No.	Diagnosis	Typical fish pattern N=239	Atypical fish pattern N=130
1.	CML-CP	190 (70.3%)	80 (29.7%)
2.	CML-CP with increased fibrosis	10 (34.5%)	19 (65.5%)
3.	CML-BC	33 (61.1%)	21 (39.9%)
4.	CML – BC with EM	06 (37.5%)	10 (62.5%)

FISH: Fluorescence-in-situ hybridization; CML-CP: Chronic myeloid leukemia chronic phase; CML-BC: Chronic myeloid leukemia- Blast crisis

### Comparing FISH patterns between CML-CP and CML-CP with fibrosis

On comparing the FISH patterns between CML-CP and CML-CP with fibrosis [Table 3], atypical patterns were present in 65.5% of cases of CML-CP with fibrosis, while it was identified only in 29.6% of CML-CP cases. This difference was found to be significant (p-value-<0.001).

**Table 3: Comparison of FISH patterns in CML-CP and CP with fibrosis (p<0.001)**

Diagnosis	Typical	Atypical	Total
CML-CP	190 (70.3%)	80 (29.7%)	270
CML-CP with	10 (34.5%)	19 (65.5%)	29

FISH: Fluorescence-in-situ hybridization; CML-CP: Chronic myeloid leukemia chronic phase

**Table 4: Comparison of FISH patterns in CML-CP and CML-BC (p=0.007)**

Diagnosis	Typical	Atypical	Total
CML-CP	190 (70.3%)	80 (29.7%)	270
CML-BC	39 (42.6%)	31 (57.4%)	54

FISH: Fluorescence-in-situ hybridization; CML-BC: Chronic myeloid leukemia blast crisis; CML-CP: Chronic myeloid leukemia chronic phase

### MR status by RT-PCR

At the end of 12 months of therapy, out of 369 cases, RT-PCR was performed in 324 of these cases. In the remaining 45 cases, RT-PCR was not done as they were lost to follow-up. Out of 324 cases, major molecular response was attained in 132 (40%) cases at the end of 12 months of therapy, and the remaining 192 (60%) cases failed to achieve MMR.

### Correlation of morphological diagnosis with MMR status

Among CML-CP, 47.9% cases attained MMR. However, both CML-BC (22.4%) and CML-CP with fibrosis(12.5%) showed significantly lower MMR attainment (p value<0.001) [Table 5].

**Table 5: Correlation of diagnosis with MMR status (n=324) (p<0.001)**

Diagnosis	Attained MMR	Not attained MMR	Total
CML-CP	116 (47.9%)	126 (52.1%)	242
CML-BC	13 (22.4%)	45 (77.6%)	58
CML-CP with increased fibrosis	3 (12.5%)	21 (87.5%)	24

MMR: Major molecular response; CML-BC: Chronic myeloid leukemia blast crisis; CML-CP: Chronic myeloid leukemia chronic phase

**Correlation between FISH patterns and RT-PCR MMR status**

On comparison of FISH patterns with the presence of MMR on RT-PCR, out of 194 cases that had typical FISH pattern, 122 (62.9%) attained MMR while the remaining 72 (37.1%) cases failed to attain MMR. However, among the 130 cases which had atypical FISH pattern, only 10 cases (7.7%) attained MMR, and the major population of 120 (92.3%) cases failed to attain MMR. This finding was also statistically significant (p value <0.001) [Table 6].

**Table 6:** Correlation of MMR status and FISH pattern (p<0.001).

RT-PCR (N=324)	Attained MMR	Not attained MMR	Total
Typical FISH pattern	122 (62.9%)	72 (37.1%)	194
Atypical FISH pattern	10 (7.7%)	120 (92.3%)	130

MMR: Major molecular response; FISH: Fluorescence-in-situ hybridization; RT-PCR: Reverse transcription polymerase chain reaction

EUTOS score:

**Correlation between EUTOS score risk stratification and MMR status**

Among 266 cases of CML-CP in which RT PCR was performed, in 204 high-risk cases, 42% (N=85) cases attained MMR. However, in the 62 low-risk CML-CP cases, 55% (N=34) cases attained MMR, which was significantly higher (p value <0.05) [Table 7].

**Table 7:** EUTOS score risk stratification and MMR status (p < 0.05)

EUTOS Risk	Attained MMR	Not attained MMR	Total	Attained MMR %
Low risk	34	28	62	55%
High risk	85	119	204	42%
Total	119	147	266	

EUTOS European Treatment and outcome study score; MMR: Major molecular response

**Table 8:** FISH patterns with EUTOS score risk stratification

FISH patterns	Low EUTOS risk	High EUTOS risk	Total	Low EUTOS risk %
Typical	44	123	167	26.3
Atypical	18	88	99	18.2
Total	62	204	266	

FISH: Fluorescence-in-situ hybridization; EUTOS: European Treatment and outcome study score

**Correlation of FISH patterns with EUTOS score risk stratification and MMR status**

Out of 266 CML-CP cases in which RT-PCR was performed, there was no significant difference in EUTOS risk between typical and atypical FISH patterns (26.3% and 18.2% of low EUTOS risk in typical and atypical FISH patterns, respectively) [Table 8]. However, there was a significant difference in MMR attainment risk between typical and atypical FISH patterns. 65.9% CML-CP cases with typical FISH patterns attained MMR, while only 9.1 % of cases with atypical FISH patterns attained MMR (p value <0.001) [Table 9].

**Table 9:** FISH patterns in CML-CP and MMR (p<0.001)

FISH patterns	Attained MMR	Not attained MMR	Total	Attained MMR %
Typical	110	57	167	65.9%
Atypical	9	90	99	9.1%
Total	119	147	266	

FISH: Fluorescence-in-situ hybridization; MMR: Major molecular response; CML-CP: Chronic myeloid leukemia chronic phase

**DISCUSSION**

Myeloproliferative neoplasm (MPN) is a heterogeneous group of disorders characterized by abnormal proliferation of one or more terminal myeloid cell lines in the peripheral blood and bone marrow. It accounts for one-fifth of all adult leukemia cases.<sup>[6,7]</sup> Conventional cytogenetics is the most commonly used method for confirming the diagnosis. The role of FISH has increased in recent times as it can detect the presence of cryptic or variant translocations that cannot be detected by conventional karyotyping. Also, it gives additional information regarding the signal pattern, whether it is typical or atypical.<sup>[4]</sup>

Although the extensive use of tyrosine kinase inhibitors like imatinib significantly reduces disease progression and thereby increases disease-free survival, relapse and drug resistance remain an unsolved issue. Hence, monitoring the treatment response is essential using the RT-PCR technique.<sup>[8]</sup>

In this study, we have studied the typical and atypical FISH patterns in CML. Also, we have studied the disease course of atypical FISH patterns by comparing them with the typical ones and the subsequent monitoring of those patients' molecular response through RT-PCR. We have tried to detect and predict the disease progression in CML cases with variant signal patterns and assessed whether it might

be an effective way to provide prognostication and treatment choices for such patients. Ours is the first Indian study comparing atypical FISH patterns in CML with morphology and molecular response.

### Baseline characteristics

In our study, 369 cases of CML were reported during the period of 18 months. The median age at presentation was 41 years. Most of our patients presented between 40-60 years of age. This is concordant with many previous studies and review articles.<sup>[9-11]</sup> The youngest presenting age was 9 years in our study. Male to female ratio in our study was 2:1. This is also in concordance with the available literature, which stated that males are more likely to be affected than females.<sup>[12]</sup>

### Morphological subtyping

The 2022 WHO Classification adopted a biphasic scheme for the natural history of CML by only recognizing the chronic phase (CP) and Blast phase (BP) with an intermediate group called high-risk chronic phase.<sup>[12,13]</sup> Many literature studies stated that approximately 85% of people are in the chronic phase of CML.<sup>[14]</sup> Similarly, in our study, morphological subtyping was done in all 369 cases, and it revealed that 81% of cases were in the chronic phase and 19% of cases were in blast crisis.

Bone marrow biopsies among the CP cases were examined for fibrosis, which revealed that 29 cases (9.7%) had increased fibrosis. BM fibrosis occurs in 40% of patients with CML at diagnosis.<sup>[15,16]</sup> Bone marrow fibrosis (grade 2 or more) at diagnosis of CML, or its later development during the course of the disease was regarded as a poor prognostic factor. However, according to recent WHO updates, the degree of bone marrow fibrosis in CML does not have a bad prognosis when treated with imatinib.<sup>[17]</sup>

Extramedullary involvement (cutaneous/ bone/ lymph node/ CSF/ other body fluids involvement) has been reported in only 04% to 16% of cases of CML-BC.<sup>[18]</sup> In our study, 16 cases (23%) had extramedullary(EM) blast proliferation out of 70 cases of blast crisis.

### FISH patterns

FISH was performed in all 369 cases, and it revealed 65% of cases had typical FISH patterns, and 35% of cases showed atypical FISH patterns. This was roughly similar to the previous reported incidence of 71%-88%.<sup>[19]</sup> Atypical FISH patterns represent small deletions within the BCR and ABL1 hybridization sites and some adjacent DNA sequences, and also duplication of the Philadelphia chromosome.<sup>[20,21]</sup>

In this study, we found 9 atypical FISH patterns apart from the typical one (1O1G2F). The most common atypical

FISH pattern was 1O1G1F. The majority of chronic phase CML cases showed typical FISH patterns (70.3%), whereas 57.4% of the blast crisis cases showed atypical FISH patterns in our study, thus indicating a more aggressive course of presentation. 65% of the CML cases with fibrosis showed atypical FISH patterns while only 29.7% of CML cases without fibrosis showed atypical FISH patterns. Also, among the extramedullary BC cases, 62.5% cases showed atypical FISH patterns. From these findings, our study has proven that there is a significant difference between CML-CP, CML-BC-BC- BC and CML-CP with fibrosis with respect to the type of FISH pattern. Atypical patterns were found to be far more common in CML-BC and CML-CP with fibrosis ( $p=0.007$  and  $p<0.001$ , respectively) than in CML-CP without fibrosis.<sup>[22,23]</sup> Overall, CML cases with atypical FISH patterns showed faster progression to advanced phases with unfavourable outcomes.<sup>[24,25]</sup> There are only very few studies that highlighted the impact of atypical FISH patterns with disease outcome. Our study is one of the few studies which mainly focused on the importance of detecting atypical FISH patterns and their correlation with various phases of CML. This highlights that any case with variant BCR-ABL1 fusion warrants a close follow-up, and a normal or noncomplex karyotype detected in these cases by conventional karyotyping is misleading in terms of risk stratification, response categorization, and clinical follow-up.

### Determination of molecular response through RT-PCR

RT-qPCR quantifies the amount of BCR-ABL1 mRNA in the blood, thereby allowing precise measurement of leukemic burden. The results were expressed as the ratio of BCR-ABL1 to ABL1 (housekeeping gene) and expressed on the International scale (IS). In our study, MMR was evaluated using qRT-PCR at the end of 12 months of therapy with TKIs with or without chemotherapy. Out of 369 cases, 45 cases were lost to follow-up. In the remaining 324 cases, 40% cases attained MMR and 60% failed to achieve MMR. Several studies have pointed out that achieving MMR early is associated with better long-term survival and a low risk of disease progression.<sup>[26,27]</sup> IRIS Study (International Randomized Study of InterferonVs. STI571) demonstrated that approximately 40% of patients treated with imatinib achieved MMR at the end of 1 year, similar to our study.<sup>[28]</sup> A study by Zhimei Cai *et al.*, based on molecular evaluation of 79 cases of CML, showed that 51.9% cases achieved MMR at the end of 1 year of imatinib therapy.<sup>[29]</sup>

### Correlation of Phases of CML and EUTOS Risk Stratification With MMR Status

In our study, the percentage of CML cases in chronic phase that attained MMR was significantly higher than those in blast phase and CML with increased fibrosis. 48% of CML-CP attained MMR out of 324 cases, whereas only 22.4 %

and 12.5% cases of BC and CML with increased fibrosis attained MMR, respectively. These differences were clinically significant ( $p < 0.001$ ). This also demonstrates that, with disease progression (either fibrosis or blast crisis), the time required to achieve a major molecular response increases.<sup>[30]</sup> Few studies have also pointed out that patients diagnosed with chronic phase CML, who have started therapy early, have a better prognosis and a higher probability of achieving durable molecular responses than those in advanced phases.<sup>[31]</sup> We also observed that 55% of low EUTOS risk cases attained MMR while only 42% of high-risk cases attained MMR, which was significant ( $p < 0.05$ ), and this finding corresponds with previously published literature.<sup>[2]</sup>

#### Association Between FISH Patterns and RT-PCR Status

While comparing the FISH patterns with MMR status, 70% of cases with a typical FISH pattern attained MMR, but only 7.7% of cases with an atypical FISH pattern attained MMR at the end of 12 months. This significant difference in molecular responses might be attributed to increased complexity of chromosomal abnormalities, such as additional clonal evolution leading to a more aggressive disease course and resistance to standard therapies, with an overall poor prognosis. Even though a detailed analysis comparing atypical FISH patterns with molecular response has not been performed till now, a few of the studies have stated that atypical FISH patterns are linked to slower achievement of molecular response.<sup>[19,32]</sup> A study by Zhanglin *et al* compared heterogeneous BCR-ABL1 signal patterns in 270 cases of CML with leukemic clonal evolution. This study showed that 52.9% cases with an atypical FISH pattern presented in blast phase were associated with additional clonal evolution and failure to molecular milestones, similar to our study.<sup>[4]</sup>

Even though CML-CP cases with typical and atypical FISH patterns did not show a significant difference in EUTOS risk assessment, they showed a significant difference in attainment of MMR. A significantly higher proportion of cases with typical FISH patterns attained MMR compared to cases with atypical patterns (65.9% vs 9.1%). This strongly suggests that FISH patterns are independently associated with treatment response, irrespective of EUTOS score. Further studies on a large scale are needed for validation of this finding.

Recent studies have shown that there is no significant difference in prognosis between CML-CP and CML-CP with fibrosis.<sup>[33]</sup> However, our study proves that CML-CP with fibrosis is significantly associated with atypical FISH patterns and also associated with delayed achievement of MMR compared to CML-CP. Further studies with long-term follow-up are required to assess the prognosis and long-term survival among cases with CML-CP with fibrosis.

Our study has also proven that atypical FISH patterns in CML

are significantly associated with faster disease progression and are less likely to attain MMR by one year than typical FISH patterns. From the above findings, it can be concluded that CML cases with atypical patterns need a closer follow-up to monitor disease progression and have a worse prognosis with significantly delayed attainment of major molecular response (MMR) compared to CML cases with typical FISH patterns.

Further studies are required to assess the response of CML cases with atypical patterns when treated with the next generation of TKIs and also to assess any change in treatment protocol required in these cases to evoke a better response.

**Author contributions** US: Design, drafting of manuscript, acquisition of data; KD: Drafting of manuscript, analysis of data; PW: Study design, critical revision of manuscript; SJG: Technical supervision; statistical analysis; PP: Study design; BP: Technical supervision; PT: Technical supervision; DMP: Technical supervision.

#### CONCLUSION

Ours is one of the few studies that analyzed and compared FISH patterns with morphological diagnosis. Also, ours is the first study to compare FISH patterns with MMR on RT-qPCR in a large population. CML BC (57.4%) and CML-CP with fibrosis (65%) significantly show more atypical patterns compared to CML-CP (29.7%). In addition, CML BC (22.4%) and CML-CP with fibrosis (12.5%) attained MMR significantly in fewer numbers compared to CML-CP (48%). Also, CML cases with atypical patterns attained MMR significantly in fewer numbers (7.7%) compared to CML cases with typical patterns (62.9%). Independent of EUTOS risk stratification, CML-CP cases with atypical FISH patterns showed a significant difference in the attainment of MMR compared to CML-CP cases with typical patterns (9.1% vs 65.9%). Further larger-scale studies to assess treatment response are required in CML cases with atypical FISH patterns.

**Ethical Approval:** The Institutional Review Board has waived ethical approval for this study. Waiver number- IRC/2025/P-95.

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