Discussion

9; 22

BCR/ABL
The discovery of the Philadelphia chromosome as a hallmark of CML in 1960 by Nowell and Hungerford provided an evidence for a genetic link to cancer. As with most seminal scientific observations, the description of the Philadelphia chromosome posed many more questions were answered. Hence the first spectacular success in cancer cytogenetics came with invention of Ph chromosome. The confusing plethora of karyotypic aberrations encountered in other malignancies suggested that the chromosomal changes were epiphenomena incurred during tumor progression rather than essential early pathogenetic factors. The new methodology completely revolutionized cytogenetic analysis. The perceived significance of the Ph chromosome also changed, and the very uniqueness of the marker came to be regarded as a perplexing oddity. Soon afterward, Ph was characterized and the nonrandomness of karyotypic changes was also demonstrated beyond doubt in specific types of human hematologic disorders and solid tumors.

Consequently, cytogenetic analysis is established as an invaluable tool in the diagnosis, prognosis and management of hematological malignancies. It is most way to detect the whole genome. Our laboratory is the single major center in Gujarat, India that analyze not only large no of patients with hematological malignancies for conventional cytogenetics but also offers various molecular cytogenetic assessment such as FISH, M-FISH, BAC-FISH, RT-PCR etc. In CML the impact of cytogenetic abnormalities are precisely defined in the role of diagnosis, prognosis and in therapeutic strategies. The present series included total 393 CML patients to know the significance of cytogenetic studies. 361 patients were enrolled at the time of diagnosis and 32 patients were already treated with anticancer therapy at the time of enrollment for this study.

**Etiology and Epidemiology in the present study:**
Any precise etiological factor for CML with any of the patients was not observed in the present study. Out of 393 patients, 235 (60%) were males and 158 (40%) were females. Male to female ratio in the present study was
3M:2F which showed slight male predominance, which is in accordance with previous reports (Turhan, 2008).

![Figure-104: Age distribution of CML patients in present series.](image)

The reported median age range of CML patients is 30-60 years worldwide (Turhan, 2008). In India, the median age at onset is 38-40 years as reported by Farah and Kanjaksha, (2007). In the present study, the patients presented a wide age range [Figure: 104]; i.e. 4-76 years. The higher incidence of CML was observed in the age range 31-40 and 21-30 years which accounted for 131 (33%) and 89 (27%) patients, respectively. The median age was 36 years. The age range for male patients was 4-76 years with median age of 36 years. The age range for female patients was 7-76 years with median age of 37 years. CML in pediatrics is rare and constitutes only approximately 2% of all childhood leukemias (Suttorp et al, 2008). In the present study there were 11 (3%) pediatric patients out of 393 patients. Out of 11 patients, 10 patients were in chronic phase and 1 patient was in blast crisis at diagnosis.
**Ph assessment:**
Various investigations have conclusively established that, at diagnosis more than 90 patients show Ph chromosome. This is usually the sole cytogenetic anomaly. In the present study, 344 (95%) patients were Philadelphia positive. 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 analyses. In present assessment, total 13 (4%) patients showed masked Philadelphia. 9 patients showed normal karyotype, 2 patients were involved in variant three-way translocations and 2 patients showed complex chromosomal rearrangements. These patients were further characterized by molecular cytogenetic methods. 4 (1%) patients were Ph negative- $BCR/ABL$ negative with other abnormality in the present study. These patients were characterized as atypical CML. It is a chronic myeloproliferative disorder with a clinical and hematological picture similar to CML but lacking Ph chromosome and $BCR-ABL$ rearrangements (Hernandez et al, 2008).

**Categorization of cytogenetic abnormalities:**
Earlier studies on CML mainly consisted of smaller series on different aspects viz. variants or secondary changes or complex or masked Ph etc., Recently, Albano et al (2010) presented a group wise series of 452 patients. The study was divided into three groups. From India, a large no. of patients with CML was analyzed by Jacob et al (2002); the study was carried out only conventional cytogenetics. Jha et al (2006) documented data of 35 CML patients. Chavan et al (2006) reported 175 patients studied with only conventional cytogenetics. No such series is recently available with all aspects including latest molecular paraphernalia with targeted therapy outcome in correlation with cytogenetic studies. The present study, 393 patients were subgrouped into 7 categories describing different aspects of cytogenetics including latest molecular tools and therapy.
Category 1: Classical Philadelphia (Sole) (311 patients):

During the initial indolent chronic phase of CML, the Ph chromosome is usually the sole cytogenetic anomaly (Orciuolo et al, 2007). Sole Ph was observed in 311 (79%) of the patients in the present study. Total 24 patients were in advanced phase (17 patients-acute and 7 patients-blast crisis) and remaining 287 patients were in chronic phase.

In typical translocation cases, del(9q) occur in (15%) of cases (Bennour et al, 2009). It has been suggested that deletions of derivative chromosome 9 account for the inferior survival of patients with variant Ph translocations (Sinclair, 2000). Huntly et al (2001) reported a major cytogenetic response rate in patients with 9q34 deletion and treated with Imatinib in chronic phase, as compared to patients without deletion. Recent studies (Soenen et al, 2008) do not support the previous hypothesis regarding with deletion 9q and poor prognosis in CML patients. These studies demonstrated that the response rates and survivals were similar to those of patients without deletion 9q. Thus, they suggested that Imatinib should be considered in the same way as it is used for all patients with Ph-positive CML. Present study performed a DCDF-FISH for BCR/ABL in 58 patients, of which 40 patients showed typical positive signal pattern for BCR/ABL fusion. 8 (31%) patients showed variant signal patterns. No clonal evolution was observed amongst variants. Among variants [Table: 5], deletion on der(9) was observed in 16 (27%) patients.

Out of 16 patients with deletion 9q, 13 patients showed complete hematologic response. Thus present study also supports the previous hypothesis that, patients with deletion (9q) show inferior therapy response. Out of 18 patients, 15 (83%) patients showed complete hematologic response, whereas 3 (17%) patients failed to achieve hematologic response. 9 patients showed complete cytogenetic response, response was not achieved in 5 patients.

Out of 18 patients, 2 (11%) patients showed 2R2G1F signal pattern. Of these 2, one patient showed two-step variant mechanism involving der(11p15) [Figure: 50]. The translocation of ABL/BCR on short arm of der(11) was
cryptic, hence not revealed by conventional cytogenetics as well as WCP FISH. The reasonable explanation is that part of the \textit{ABL} gene which took part in translocation was so small beyond the sensitivity of conventional cytogenetic and painting FISH, hence only DCDF provided a clue on invert DAPI image \[\text{Figure: 50}\]. The t(9;11;22) involving 11p15 region have been reported as one-step mechanism (Richebourg et al, 2008). However, present study found such rearrangement with two-step mechanism with different signal pattern, which is a rare event. The three way t(9;11;22)(q34;p15;q11.2) is reported only twice (Morel et al, 2003). The involvement of 11p15 is rare in CML (Mitelman, 2010). The 11p15 region contains \textit{NUP98} gene, which is correlated with poor prognosis. In CML with blast crisis, involvement of \textit{NUP98} gene is observed with the t(7;11) as the most common secondary change. It involves \textit{NUP98/HOXA9} genes. The aberrant expression in context of these fusion proteins causes increased proliferation in the case of \textit{NUP98/HOXA9} (Radich, 2007). Conversely, the patient in present study achieved complete hematologic and cytogenetic response. Thus, the involvement of 11p15 neither showed disease progression nor affected disease outcome.

In 80-85\% of patients, the evolution of CML from chronic to acute and eventually into blast crisis is observed. This evolution is accompanied by the developments of subclones of the initially transformed cells with new cytogenetic and molecular changes, in addition to the Ph (Orciuolo et al, 2007). The frequency of this phenomenon is referred as clonal evolution (Falchi et al, 2010). Clonal evolution after treatment was observed by various researchers (Cortez et al, 2003). 12.3\% of the patients developed clonal cytogenetic evolution during treatment as presented by Schoch et al, 2003. In our study, 10\% of the patients developed clonal evolution, which is similar to the results with other studies (Schoch et al, 2003). In this category, total 31 (10\%) patients \[\text{Table: 6}\] showed clonal evolution. The extent to which cytogenetic abnormalities occur and may affect prognosis in patients treated with Imatinib has also been studied. Clonal evolution can indeed occur also during treatment with Imatinib. Acquisition of clonal evolution increases the
Discussion

risk of subsequent disease progression also in CML patients in complete hematologic response on Imatinib (Sarah et al, 2003). The most common major cytogenetic changes seen in CML in the advanced phase include a +Ph, +8, i(17)(q10), and +19 (Orcicuolo, 2007). Present study revealed major and minor route cytogenic changes in 21 patients [Figure: 17]. In the present study also major route changes were the most common i.e. +8 (11 times), +Ph (8 times), i(17)(q10) (4 times) and +19 (3 times). The minor route cytogenetic changes were observed in less frequency. It included monosomies of chromosomes 7, 17, and loss of Y; trisomies of chromosomes 17, 21; and translocation t(3;21)(q26.2;q22) [Figure: 17] (Johansson B, 2002; Reid, 2008). The minor route changes were less frequently observed than major route changes i.e. at once only including +17, +21, and loss of Y.

It is to comprise and document most of the major and minor cytogenetic alterations together are such reports are very rare. Orcicuolo et al (2007) and Lu and Wang (2007) have reported concomitant insurgence of two (+8 and i(17q10)) and three (+8, +Ph and i(17q10)) additional cytogenetic aberrations in addition to Ph. Here, we also report such concomitant abnormalities in addition to Ph [Table: 6]. The case nos. 7 and 29 showed, +8 and +19 and both the patients showed complete hematologic response. The cytogenetic response was not achieved in case 7 and case 29 showed partial response. The case nos. 20 and 23 showed, +8 and +Ph, both failed to achieve cytogenetic response. The hematologic response was achieved in case no. 23. The case no. 16 showed i(17q10) and +Ph, and no response was achieved. The case no 13 showed three additional changes, +8, +19 and i(17q10) and showed no response. Various researchers have considered the presence of additional chromosomal abnormalities as the sole criterion for the disease acceleration (O’Dwyer et al, 2004; Orcicuolo et al, 2007; Lu and Wang, 2007). O’Dwyer et al (2002) observed that patients with clonal evolution before therapy showed excellent response to Imatinib mesylate. However, they later reported (O’Dwyer et al, 2004) cytogenic clonal evolution as the main prognostic factors for relapse in patients even having the chronic phase.
In the present study, other cytogenetic changes in addition to Ph were observed in 4 patients [Table: 7]. \( t(3;5) \) (case no. 1) [Figure: 51-A] is a very rare translocation in CML and observed only for 3 times till date (Mitelman, 2010). \( \text{add}(Y)(p?) \) [Figure: 51-B] was a novel aberration (Mitelman, 2010). The marker chromosome was observed in case no. 21 [Figure: 51-C]. The case no 17 showed hyperdiploid (2n+) plates [Figure: 51-D]. Polyploidization is very rare in CML Espinoza et al (2004) detected polyploidization in 35.3% patients in their study. Moreover, polyploidization has been reported in several cell lines positive for \( BCR/ABL \) fusion (Drexeler et al, 1999). Polyploid cells develop from diploid progenitors via successive rounds of DNA synthesis without cell division, a process termed endomitosis (Espinoza et al, 2004). There is no direct evidence that the \( BCR/ABL \) protein affects such process. However, it has shown that RAS and MAPK are activated by the \( BCR/ABL \) protein. This could promote endomitosis through the MAPK pathway (Cortez et al, 1997). Polyploidization may render a more aggressive phenotype of tumor cells by facilitating drug resistance and promoting the evolution of the tumor (Espinoza et al, 2004). This patient (case no. 17) showed no response which is in agreement with the observations reported by Espinoza et al (2004).

2 patients showed other cytogenetic changes in addition to Ph and major route changes i.e. \( \text{add}(6p) \) (case no. 3) with +Ph. This additional change is very rare; patient is on Glivec till date and achieved complete hematologic response. Second patient showed \( \text{add}(2q) \) with +8 and +21 (case no. 24) [Figure: 52-A, B]. \( \text{add}2q \) is also a rare cytogenetic abnormality (Mitelman, 2010). In this patient, minor and major cytogenetic changes were observed. The patient was treated with Glivec and showed no response. In this case, trisomy 21 a rare minor route change was observed. Recently, Lestienne et al (2008) have showed that \( RUNX1 \) DNA-binding mutations and \( RUNX1-PRDM16 \) cryptic fusions in leukemia are frequently associated with secondary trisomy 21 and may contribute to clonal evolution and Imatinib resistance.
One pediatric patient showed CCR on clonal evolution (case no. 31). The CCR is related with high genomic instability. Patients with CCR may have poor prognosis (Babicka et al, 2006). This patient was treated with Glivec and survived for 2 year. However, the patient expired during the course of treatment [Figure: 52-C]. It suggested that high genomic instability due to CCR is indicative of poor prognosis.

Recently, a few chromosomal abnormalities including trisomy 8, loss of Y and monosomy 7 have been reported to occur in the Ph-ve cells during Imatinib therapy (Kim et al, 2008). As in past studies, trisomy 8 was the most common cytogenetic abnormality observed in Ph-ve cells of CML patients treated with Glivec (Kim et al, 2008). In a previous study, transient phenomenon of trisomy 8 was reported in 1.5 % patients (Kim et al, 2008) however, Lin et al (2006) reported in higher frequency. It is reported that it is not related to disease progression. In present study after post-treatment follow-up, trisomy 8 was encountered in 3 (1%) Ph-ve patients. All the 3 patients showed complete hematologic and cytogenetic response; hence the trisomy 8 seems to be transient as described earlier (Kim et al, 2008).

**Category 2: Secondary chromosomal changes (13 patients):**

Additional chromosomal abnormalities are reported in 10-20% of patients presenting chronic phase. These additional chromosomal abnormalities are reported in 30% of patients developing accelerated phase features. These have been associated with poor prognosis (Kadam et al, 1991; Block, 1999; Orcicuolo et al, 2007). Karyotype abnormalities in addition to Ph, has diagnostic, prognostic and therapeutic significance. These abnormalities are associated with acceleration of the disease and have also play a role in CML resistance to Imatinib (Hochhaus et al, 2003). The most common major cytogenetic changes seen in CML with advanced phase include; +Ph, +8, i(17)(q10), and +19 (Orcicuolo et al, 2007). The Minor route cytogenetic changes include monosomies of chromosomes 7, 17, and loss of Y; trisomies of chromosomes 17, 21; and translocation t(3;21)(q26.2;q22) [Figure: 17] (Johansson et al, 2002; Reid et al, 2008). In this category, at disease onset;
total 13 (3.3%) patients showed secondary chromosomal anomalies in addition to Ph [Table: 7]. Out of 13 patients, 10 patients were in chronic phase; whereas, only 3 patients were with blast crisis phase. The trisomy 8 was observed in 50% of the patients, which is higher in comparison to previous study i.e. 34%. In literature, +19 is observed in only 13% whereas, in the present study it was higher i.e. 33.3%. The +Ph in a previous study showed in 30% and in present study it was in 25% of the patients. The structural anomaly i(17)(q10) is seen 20% in literature and in present study it was 16.6% of the patients. The loss of Y and +21 were reported earlier (Johansson et al, 2002) 8% and 7%, respectively were comparable with 8.3% for both numerical changes in the present study.

As described in category 1, additional chromosomal alterations together are very rarely documented. In this category also, concomitant abnormalities in addition to Ph were observed. These abnormalities are described in table: 8. +8 and i(17q10) were present in case 2., +8, i(17q10), +19 and were observed in case 8. +8, +19 and +21 were observed in case 9. Case 12 showed +8, +Ph, +19 and structural abnormality involving p arm of the chromosome 17, which may indicate involvement of p53 gene. Involvement of these four major changes is not previously reported in a single case. The unusual karyotypes were also observed in 3 patients. The patient no.11 showed t(2;8)(p?24;q?13) in addition to Ph, which is rare anomaly and reported only twice in literature till date (Mitelman, 2010). It was secondary to Ph as FISH results showed typical positive signal pattern for BCR/ABL with DCDF probe [Figure: 54]. The case no. 12 showed +der(17)?t(10;17)(p14;p13) in addition to other major route cytogenetic changes, which documented that other cytogenetic changes may occur with common aberrations. The third patient (case no.13) showed inv(2) in addition to Ph. The inv(2)(p23q35) is very rare, difficult to identify, as breakpoints lie in telomeric regions. It is not well documented in CML. Till date only 4 cases are reported in the literature in CML (Huret, 2001; Mitelman, 2010).
The occurrence of additional chromosomal changes may also have a prognostic value. The occurrence of additional non-random chromosome abnormalities during the course of the disease has been extensively described in the past and considered an unfavorable prognostic factor, particularly when belonging to the major route type (Mitelman et al, 1993; Sarah et al, 2003). A few studies deal with the outcome of patients who show additional chromosomal changes at diagnosis. Imatinib treatment has greatly modified the course of Ph-positive CML. Nevertheless, resistance to Imatinib is associated with well-defined chromosomal and molecular changes (Hochhaus et al, 2002; Druker, 2008). Information regarding the fate of the subgroup of patients carrying additional chromosomal changes at diagnosis is still lacking. Out of 5 patients treated with Glivec in present study, 4 patients showed complete hematological response however, no cytogenetic response was achieved in any of the patients. 2 patients case no. 8 and 12 were expired within 3 and 6 months, respectively with major route change and other cytogenetic changes, which shows that additional chromosomal changes are predictive of poor prognosis. In these two patients concomitant additional changes were also observed. Thus, all additional cytogenetic alterations together are indicative of poor prognosis. Patients died before they could achieve therapeutic response.

**Category: 3 Three-way translocations or variants (16 patients):**

Variant Ph translocations occurring in patients with CML have been recognized for more than 20 years and have been reported in 5-10% of CML patients. Masked Ph chromosomes can be found in cases with a normal karyotype, as a result of a cryptic rearrangement or in patients with complex changes. In such cases typical t(9;22)(q34;q11.2) is not detectable by conventional cytogenetics (Morel et al, 2003; Babicka et al, 2006). However, descriptions have been based on case reports or small series. Thus, the clinical course and influence of these variants on long-term outcome is poorly understood, and conclusions are conflicting. Several studies have suggested that patients with variant Ph translocations may have an adverse prognosis, but others have suggested that these translocations have no effect of prognosis (Bennour et
In the present study, 16 (4%) cases with variant three-way translocations were analyzed. Out of 16 patients, 2 patients (case no 5 and 8) showed masked Ph with other abnormalities. With the LSI BCR/ABL DCDF in each of 16 patients and WCP FISH in 9 patients were performed. Present study with different FISH probes, elucidated the mechanism for the formation of variant translocations. The mechanism of the formation is controversial. Many favor the one-step mechanism, wherein chromosome breakage occurs on three different chromosomes simultaneously in a three-way translocation, for instance, and reciprocally rejoin at the same time [Figure: 19-a]. Others proposed a two-step mechanism, in which a standard two-way t(9;22) translocation is followed by subsequent translocations involving additional chromosomes [Figure: 19-b]. The two-step mechanism suggests that the formation of a variant translocation is similar to or is in essence a clonal evolution (Gorusu et al, 2007). In the present study, high prevalence of one-step mechanism was observed in 10 (62%) patients, two-step mechanism was observed in 3 (18.75%) and multiple-step was observed in 3 (18.75%) of the patients [Table: 8]. Surprisingly; out of 3 patients, 2 patients having 2-step mechanism expired within 2 and 3 months, respectively during the course of treatment. This observation is in agreement with Huret (1990) and Gorusu et al (2007) who proposed that two-step mechanism is essence a clonal evolution and may predict a poor prognosis. One patient with multiple-step mechanism [case no. 4 Table 8] showed additional chromosomal changes in addition to previous clone, which suggested that at disease onset, presence of multiple breaks and deletion of ABL/BCR on der(9) may be correlated to poor prognosis. Mechanisms of rearrangement in our cases occurred independently; they were not mixed, as described by Gorusu et al (2007).
As described recently by Bennour et al (2009); WCP FISH study is valuable in determining the details of complex variant translocations. We have performed WCP FISH in 9 patients, of which main two rearrangements observed were; translocation (case no 1, 2, 3, 7, 8, 9, 13 and 14) and insertion (case no 11). It is reported that deletions on der(9) occur more frequently in 60% CML cases with variant translocations which is higher than in typical translocation cases (15%) (Bennour et al, 2009). Present series showed that out of 16 patients, deletion on der(9) was observed in 4 (25%) cases (3, 4, 5 and 13). In the present study, similar frequency (27%) was also noted in category 1 (patients with sole Ph and del(9q)). Therefore the present study do not support that the deletions occurs more frequently in patients with variants. The 3 cases with multiple-step mechanisms showed deletion of ABL/BCR on der(9) [case no. 3, 4 and 14 Table 8]. Case no. 5 showed 3’ BCR with one-step mechanism. Numerous authors have considered that deletions on der(9) are associated with a shorter duration of chronic phase and shorter survival in patients treated with interferon therapy hence might counter adverse prognostic factors. To date, it remains controversial whether patients with a der(9) deletion have a different outcome if treated with Imatinib mesylate (Bennour et al, 2009). In the present study, all 4 patients with deletion showed no response, however; survival ranged from 1month-6 years. So, deletion accompanying with variants is a crucial event in CML patients.

It was worth mentioning about some interesting cases; the case 1 showed novel rearrangement involving Xq13 region. Study found a structurally abnormal X chromosome in 1% of abnormal hematological samples. Structural abnormalities of the sex chromosomes; X and Y are rarely found in neoplastic disorders (Babicka et al, 2006). However, translocations involving the long arm of chromosome X are more frequent. Band Xq13 may be more prone to structural rearrangement than other X chromosome bands in hematologic disorders (Dewald et al, 1989). Case 11 diagnosed as CML with blast crisis showed involvement of both the 22, one was involved in Ph and other was involved with der(9) as ABL/BCR was inserted in 22q13 region. Similar findings were reported by Richebourg et al (2008) with one-step
mechanism. Recently, Jarmuz et al (2010) also reported 11th such case with t(9;22;22) with one-step mechanism. Here, the patient showed two-step mechanism, which is not observed till date. The 22q13 region merits further study. In present study using BACs for chromosome 9 and 22 breakpoint was confirmed [**Figure: 98**].

![Figure: 98](image)

*Figure-105: Schematic representation showing steps in variant formation and clonal evolution describing in three events for case no 14.*

The case no. 14 showed very interesting cytogenetic map showing inv(9)(p13q34) along with t(9;22;17). The patient showed one-step mechanism with FISH results involving additional chromosome 17 as a first event. The inv(9)(p13q34) may be secondary event to t(9;22;17)(q34;q11.2;p13). The mechanism behind this phenomenon is depicted in **figure: 105**. We assume that in the second event, the patient lost 17p13 region which contains p53 gene, which serves the presence of i(17)(q10). The treatment outcome could not be ruled out as patient was newly diagnosed.

The frequencies of each aberrations and breakpoints were compared with Mitelman database [*Table: 13*]. Nevertheless, it became difficult to report
the exact number of cases with such complex translocations due to the large amount of variability in cytogenetics nomenclature observed before ISCN, 2005.

<table>
<thead>
<tr>
<th>Translocation (t)</th>
<th>No. of cases in Mitelman database</th>
<th>Present study: Total no. of cases/Case no.</th>
<th>Breakpoint observed in present study; no. s in Mitelman</th>
<th>Entity</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(1;9;22)</td>
<td>39</td>
<td>3/ 2, 12, 15.</td>
<td>1q42-2;1q21-5;1p32-1</td>
<td>Rare</td>
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<td>Novel</td>
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<tr>
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<td>1/ 10.</td>
<td>3p13-1</td>
<td>Rare</td>
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<td>41</td>
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<td>t(5;9;22)</td>
<td>31</td>
<td>1/ 3.</td>
<td>5q13-10</td>
<td>Rare</td>
</tr>
<tr>
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<td>Rare/Novel</td>
</tr>
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<td>7q22-5</td>
<td>Rare</td>
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<td>5</td>
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<td>--</td>
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<td>11q23-11q13-16</td>
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<td>2/ 13, 16.</td>
<td>13q34-4</td>
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*Table-13: Comparison of each aberration and breakpoint with Mitelman database.*

Total 4 novel and 12 rare three-way translocations were identified in the present study. Total 17 breakpoints were identified. 13q14 breakpoint was found to be non random as observed twice. As described recently by Bennour *et al* (2009) breaks tend to fall within the G-C (light bands of chromosome)
richest parts of the genomes. The present study also documented similar results [Figure: 57]. This correlation is not absolute, but would be consistent with the limited number of variant breakpoints in the present study and with the imprecise nature of cytogenetic breakpoint assignment. More recently, Fisher et al (2005) provided a model to elucidate incidence of breaks in variant translocations in leukemia. They proposed that “open” chromatin, which is transcriptionally active, is relatively likely to undergo breakage and repair, with a consequent tendency to illegitimate recombination and translocation. Authors suggested that breaks might show a high density of Alu elements which are condensed in GC-richest chromosome regions.

Evaluation of the prognostic significance of these translocations has been analyzed in case reports or smaller series giving controversial results. However, it has been recently reported that patients with variant translocations have a similar prognosis to those with classical Ph translocations when treated with Imatinib mesylate. In our series, out of the 16 patients, 9 patients were treated with Hydroxyurea and Glivec. Out of 5 patients with one-step mechanism; 4 patients showed complete hematological response and 1 patient showed no hematological response. For cytogenetic response with one-step, one patient showed complete cytogenetic response, one patient showed partial cytogenetic response, two patients could not achieve cytogenetic response, and one patient was lost to follow-up. In two patients with two-step mechanism, complete hematological response was achieved however the patients expired during the course of treatment. In two patients with multiple-step mechanism, no response was achieved. 4 patients were treated with only Hydroxyurea, showed no response. 3 patients were newly diagnosed hence correlation of prognosis and treatment outcome could not be evaluated. Variant Ph rearrangements involve additional chromosomes and, possibly, additional genes implicated with the BCR/ABL rearrangements. This may have prognostic implications when it occurs in the setting of clonal evolution and could adversely affect treatment outcome.
Discussion

Category 4: Complex chromosomal rearrangements (CCR) (8 patients):

CML is one of the best characterized malignant diseases. Well-Known chromosomal aberrations occur during progression and clonal evolution of the disease as described in category one and two of the present series. However, developments of CCR at diagnosis or during progression of CML have not been clearly defined due to the limitations of sensitivity of conventional cytogenetic analysis. The CCR are rather rare, and the significance and frequency of different anomalies are poorly understood (Babicka et al, 2006). In this category, study was carried out to determine the chromosomes and chromosomal regions which are involved in CCR at diagnosis of the disease. In the present series CCR were observed in 8 (2%) patients [Table: 9]. More than three chromosomes were found to be involved in the CCR. Minimum 4 and maximum 7 chromosomes were involved in CCR. Because of complicated chromosomal translocations shown by the GTG banding method, total 3 patients were further characterized by M-FISH. The revised karyotypes have revealed great additional information.

As described in category 3, masked Ph can be observed in cases with a normal karyotype, as a result of a cryptic rearrangements or in patients with complex changes (Babicka et al, 2006). In this category also, 2 patients (case no. 2 and 3) showed masked Ph with CCR. Though by G-banding analysis, no evidence of a Ph translocation was seen in this 2 patients, the DCDF FISH probe revealed normal location of the BCR/ABL and ABL/BCR fusion in case no 2. Variant location of BCR/ABL fusion was located on a der(5q13) instead of a der(22) in case no. 3. However, this patient showed complete hematologic response. The relocation of BCR/ABL fusion sequence on sites other than 22q11 represents a rare type of variant Ph translocation, frequency and clinical significance of such rearrangements remains to be investigated. The protein expression of BCR/ABL fusion sequence relocated to chromosomal regions other than 22q may be affected depending on adjacent gene(s) present at the area of insertion. An analysis of such data may help to delineate a subgroup of CML that may have different clinical course. This
information can be useful in interpretation of clinical trials for therapy in CML. More such reports with variant Ph may come into light with increasing use of comprehensive tools of cytogenetic techniques in cases.

The case no. 5, showed four-way translocation, variant signal pattern and 3’ BCR was observed on 4q21, which have suggested one-step mechanism. In this case part of the chromosome 9q34 was translocated to chromosome 22q11, forming Ph chromosome. The segment of 22q11 however, translocated to a chromosome 4q21 instead of 9q34. The segment from chromosome 4 was translocated to a chromosome 10 [Figure: 106]. To the best of our knowledge this four-way translocation is a novel and not previously described in the literature.

![Figure-106: Schema of the NOVEL four-way translocation by one-step mechanism, observed in case no 5. The four-way translocation was considered to be 46,XX,t(4;10;9;22)(q21;p15;q34;q11.2).](image)

The Mitelman database for chromosomal aberrations in cancer was searched for various CCR observed in present study (Mitelman, 2010). The search revealed that these combinations are novel in each of the patient. These CCR
were present at onset of the disease which suggested that these aberrations are originated in a stem cell. Bennour et al (2009) showed that any of the chromosomes can be involved in CCR. In the present study, no recurrent complex translocations were observed however, 16 different chromosomes were involved in the complex rearrangement with varied frequency [Figure: 74]. Involvement in structural rearrangement, besides 9 and 22, chromosomes 10, 11, 12 and 15 were the most frequent. Amongst the numerical changes +8 (case no. 6) and der(9)t(1;9) (case no. 7) were observed.

In the present series, 5 patients were in chronic phase and 3 patients were in acute phase at diagnosis. 4 patients were treated with Glivec and 2 patients showed complete hematologic response whereas 2 patients could not achieve hematologic response. Cytogenetic response was not achieved in 4 of the patients and 3 patients were lost for follow-up. The case no. 8 with involvement of 7 chromosomes expired. It may be assumed that due to high genomic instability, the patient succumbed to disease. The CCR of karyotype in CML patients are result of high genome instability of the tumor cells and they seem to represent the random chromosomal changes (Babicka et al, 2006). The present findings demonstrate very high instability of the genome of malignant cells at the chromosomal level. Precise determination of breakpoints involved in CCR can give newer dimension to understand the genetic mechanisms playing play role in progression of malignant disease.

**Category: 5 Normal karyotype (9 patients):**
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. Incidence of such normal cases is reported to be around 1% cases. These rearrangements can be revealed by FISH (Morel et al, 2003). Complex rearrangements in some cases represent secondary changes arising likely from two consecutive translocations with a total of four breaks, or may arise from multiple simultaneous breaks in some patients. The first translocation results in
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t(9;22)(q34;q11). The second translocation occurs involving a break distal at band 9q34 and another break on a third chromosome (Calabrese et al, 1994). The most frequent location of the \textit{BCR/ABL} fusion gene in CCR is 22q11.2 (Sessarego et al, 2000), but in rare variant cases \textit{BCR/ABL} is translocated on other sites. At least 21 cases (Storlazzi et al, 2002; Wan et al., 2004; Fugazza et al, 2005, Brahmbhatt et al, 2010) described in the literature showed fusion gene located at 9q34. Despite the advances made in defining translocations related oncogenes in CML, the molecular mechanisms of leukemogenesis in the Ph-negative-\textit{BCR/ABL} positive CML cases remain to be defined (Bennour et al, 2008).

In this category, 9 (2\%) patients showed normal karyotype with typical clinical features of CML [\textbf{Table: 10}]. DCDF FISH analysis revealed positive signals for \textit{BCR/ABL} fusion in 5 patients. With the help of metaphase FISH analysis, unusual localization of \textit{BCR/ABL} was observed on der(9) in 3 patients [case 1, 3 and 9, \textbf{Table: 10}]. Unusual location of \textit{BCR/ABL} gene is a rare event. From clinical point of view, a few cases reported with unusual localization of the \textit{BCR/ABL} fusion gene had a poor prognosis (Naumann et al, 2003; Todoric-Zivanovic et al, 2006; Gorusu et al, 2007). In the present study, on follow-up study, case no. 1 showed fusion for \textit{BCR/ABL} on both the chromosomes 9q34 and response was not achieved. Cases 3 and 9 were lost to follow-up, further clinical details were unavailable.

Interestingly case no. 2 showed fusion for \textit{BCR/ABL} on both the chromosomes 9q34 at diagnosis. On follow-up evaluation, case no. 1 showed fusion for \textit{BCR/ABL} on both the chromosomes 9q34. Six similar Ph-negative cases with double \textit{BCR/ABL} fusion genes on both 9q34 have been published previously; four were in chronic phase; two were in myeloid blast crisis. In agreement with hypothesis of Hagemeijer \textit{et al} (1993), we presume that the first molecular and cytogenetic event in bone marrow cells of both the patients could be the insertion of the 5’ part of the \textit{BCR} gene within or at the 5’ side of the \textit{ABL} gene on chromosome 9. The second event could be the duplication of the rearranged chromosome 9 together with the loss of normal
Discussion

Previously reported cases with two Ph chromosomes or double *BCR/ABL* fusion signals have been found in the bone marrow cells of the patients in transformation to accelerated and/or in blast crisis of CML. During transformation, the additional chromosomal abnormalities to Ph chromosome could be seen in several patients and are considered as indicator of unfavorable prognosis. The same applies for patients with double *BCR/ABL* fusion gene on both 9q34 (Verma and Chandra, 2000). Clinically, both patients in present study were in chronic phase at the time of diagnosis. Essential results were obtained by FISH with specific LSI *BCR/ABL* probe, which determined the location of fused gene on both chromosomes 9 and gave a warning of poor prognosis of the patients. It was repeatedly hypothesized, based on the course of the disease of previously published cases that prognosis of patients with two Ph chromosomes and/or *BCR/ABL* gene located on one or both chromosome 9 is generally very poor (Michalova et al, 2004). Out of six cases reported in the literature, only one received Imatinib treatment and achieved complete response (Dufva et al, 2005). Our both patients are receiving Imatinib treatment; both the patients are morphologically in chronic phase but leukocyte count was high and cytogenetic response is not achieved till date.

In the present study, out of four patients (case no. 4, 5, 7 and 8) with possibility of masked Ph, 3 patients (case no. 4, 5, and 7) showed clonal evolution in addition to Ph on follow-up. Case 4 showed +8 in Ph-ve cells and FISH results were negative for *BCR/ABL* [Figure: 86]. Case 5 showed major and minor route changes with structural rearrangement and *BCR/ABL*-DCDF on follow-up of 1 year showed 1R1G1F signal pattern, deletion of ABL/BCR observed on der(9) [Figure: 87]. Case 7 showed structural rearrangement involving chromosome 9, 10 and 22 [Figure: 89]. The case no 8 showed persistence of Ph in karyotyping and FISH during 1 year follow-up. The results clearly emphasize necessity of FISH studies in Ph-ve CML to rule out masked Ph.
DCDF FISH is usually performed to demonstrate also the presence of a second fusion signal caused by the chimeric ABL/BCR gene on 9q34 (Dewald et al., 1999) in addition to BCR/ABL. In case, the second fusion signal is absent, it indicates deletion of ABL/BCR sequences flanking the breakpoint region. Sometimes deletion of only 5′ ABL or 3′ BCR can be observed on der(9) (Lee et al, 2006). This observation has acquired important prognostic significance (Huntly et al, 2001). In the present study, FISH analysis using DCDF probe in 5 patients, case no 6 showed deletion of 5′ ABL. The patient showed complete hematologic response but could not achieve cytogenetic response. The deletion status was not confirmed in cases 1, 3 and 9 with masked Ph, as BCR/ABL signal mapped exactly on 9q34 instead of 22q11.2. Hence, the possible prognostic impact of del9q was not ruled out with DCDF probe. Tri-color FISH was carried out in case no. 2 and which showed presence of 2 aqua signals indicating that there was no deletion on der9.

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. The unusual location of BCR/ABL sequences on chromosome other than 22q11 apparently did not affect response to Imatinib mesylate. This is also observed in present series, as cases no. 3 and 9 both patients achieved complete hematologic response. However, cases 1 and 2 with two copies of BCR/ABL on both 9 chromosomes showed no response, so this could be anticipated as double Ph with overexpression of BCR/ABL. Out of 9 patients, 7 patients were treated with Glivec. 5 patients showed complete hematological response, whereas in BCR/ABL on both the chromosome 9 showed no response. Complete cytogenetic response was achieved in 2 patients, 3 patients showed no cytogenetic response and 2 patients were lost to follow-up.

Thus, the current investigation documented that patient with normal karyotype show BCR/ABL rearrangement. These patients may have atypical signal pattern of BCR/ABL rearrangement.
**Category 6: Ph negative and abnormal Karyotype (4 patients):**

aCML belongs to the myeloproliferative/myelodysplastic category of haematological disease. It is a rare disorder of old adults. No predominance of sex was observed in aCML. By cytogenetic definition, aCML cases lack in Ph and \( BCR/ABL \) fusion. It carries a poor prognosis with limited therapeutic options available. Overall 50-65% of patients show cytogenetic abnormalities. The most frequent is +8 (25%). Other changes such as -7 and del(12p) have also been recurrently observed. Other abnormalities are: idic(Xq); del(5q); t(6;8)(p23;q22); -9; del(11q); del(12q); del(15q); del(17p); t(17;20) and add(21q). No specific cytogenetic changes have been associated with aCML (Hernandez, 2008). Till date, 54 cases with aCML are reported with abnormal karyotype (Mitelman, 2010). In the present series 4 (1%) patients showed Ph negative karyotype with other abnormalities. There were 2 male and 2 female patients. 2 patients showed numerical change i.e. loss of sex chromosome and trisomy of 1, till date +1 is not described in aCML. Remaining 2 patients showed structural rearrangement i.e. add(1q) and add(9)(q34), t(12;17)(q13;q25) respectively. On FISH with \( BCR/ABL \) out of 4 patients, 3 patients were \( BCR/ABL \) negative. The patients were lost to follow-up within a range of 2-50 days.

**Category 7: Previously treated patient (32 patients):**

The cytogenetic studies are also helpful to know the prognosis and treatment monitoring, even if previous cytogenetic studies are not available. For this study, 32 patients who already received anticancer therapy were also enrolled [Table: 12]. After treatment, normal karyotype indicates complete cytogenetic response. In the present group, total 7 patients showed normal karyotype. In 14 patients sole Ph was present, which suggested no cytogenetic response. 5 patients showed mix clone of Ph-ve and Ph+ve cells. Clonal evolution was observed in 6 patients. It is documented that clonal evolutions indicate the disease progression and patient may develop most common cytogenetic abnormality or other cytogenetic changes (Holzerova et al, 2009). Major route changes were present in 4 patients in the present study (case 1, 4, 8, 14, Table: 12). The remaining 2 patients showed additional
changes in addition to Ph i.e.?add(7p) and del(3p14) (cases 7 and 24, Table: 12) respectively. The case no. 7 showed unusual location of BCR/ABL on der(9) with additional chromosomal anomaly add(7)(p).

Out of 32, 29 patients were treated with Glivec. The hematological response was achieved in 21 (72%) patients, of which one patient showed clonal evolution. 8 (28%) patients could not achieve hematological response of which three patients showed clonal evolution. The complete cytogenetic response was achieved in 11 (38%) of the patients. Partial cytogenetic response was achieved in 2 (7%) patients. 1 (3.5%) patients showed minimal response. 1 (3.5%) patient was lost to follow-up for cytogenetic study and 14 (48%) patients showed no cytogenetic response. Recently, Holzerova et al (2009) reported that, majority of the previously treated Ph+ CML patients benefit form starting Imatinib mesylate therapy, in contrast patients with complex karyotype have poor prognosis even with Imatinib mesylate. Out of 6 patients having additional chromosomal changes, only one patient showed complete hematologic and cytogenetic response. 2 patients could achieve hematologic response but could not achieve cytogenetic response. 3 patients showed no response even with Imatinib mesylate therapy.

**Total major and minor route cytogenetic changes:**
The major and minor cytogenetic genetic changes are most commonly seen in CML. The frequency and significance of major and minor route cytogenetic changes are well established (Johansson B, 2002, Calabretta and Perrotti, 2004; Radich, 2007). Several major and minor cytogenetic genetic changes were encountered in present study. The frequency of each aberration was compared with earlier reports [Figure: 107]. The highest change was trisomy 8 observed for 39% more or less similar to the literature i.e. 39%. The second most aberration was double Ph or +Ph or extra Ph observed in 23%, which is less frequent than described by previous investigations i.e. 30%. The vast difference was observed for i(17)(q10) i.e. 11 % in present study and which is also less as compared to literature i.e. 20%. The
Discussion

frequencies of rest of the aberrations were in accordance with earlier findings. Monosomy of chromosome was not observed in the present study.

Figure-107: Comparison of major and minor cytogenetic changes with (Johansson B, 2002) literature.

**Overall impact of different FISH strategies in present studies:**
The FISH with *BCR/ABL* DCDF was performed at diagnosis in 95 patients, out of which 3 patients showed negative results and 92 patients showed positive results for *BCR/ABL* fusion. Out of 92, 47 patients (51%) showed typical positive signal pattern, whereas, 45 patients (49%) showed atypical signal pattern. Out of 45 with atypical signal pattern, 23 (51%) patients showed del on der(9). In 9 patients with masked Ph, FISH was useful for detection of *BCR/ABL*. Different variants were analyzed by FISH (category 3, 4, 5 and 7). WCP FISH also provided a clue of mechanism of variants. We emphasize that WCP and LSI *BCR/ABL* DCDF probes were valuable in determining the details of complex variant translocation observed in these cases. The *BCR/ABL*-ES FISH was also carried out in patients with unusual localization of *BCR/ABL* fusion as DCDF probe detects two fusions. *BCR/ABL* ES FISH probe map is different and detects only *BCR/ABL* fusion hence localization of *BCR/ABL* can be assured. The Tri-color FISH was carried out and showed no deletion in one patient. FISH analysis was helpful in disclosing masked Ph chromosome, which is beyond the resolution power of conventional cytogenetics. These observations again underlined the importance of metaphase FISH to avoid erroneous interpretation of interphase FISH only as emphasized by our results (Brahmbhatt et al, 2010). Although interphase FISH is increasingly used for
BCR/ABL gene rearrangement identification in CML. Our observations and earlier reports by Lim et al (2005) and Vargas et al (2009) strengthen the fact that exact interpretation of any atypical interphase FISH pattern is dependent on FISH metaphase studies.

**Impact of M-FISH:**

Multicolor FISH (M-FISH) techniques have greatly enhanced the resolution of conventional cytogenetic analysis, thus enabling the identification of novel regions of rearrangement in hematological malignancies. M-FISH allowed a more precise description of chromosomal aberrations, the finding of cryptic rearrangements, characterization of markers, identification of additional material and a better interpretation of complex aberrations (Brizard et al, 2004). M-FISH can refine CCR and find out or correct the missed or misidentified abnormalities by conventional cytogenetics (Zhu Y et al, 2007). In the present study, M-FISH was carried out which was very helpful to demonstrate the mechanism underlying CCR and also provided additional information. In category 3; case 3 (Table: 9) showed only t(5;10) and add(22q). M-FISH was performed and results revealed that, t(5;10) was actually insertion of chromosome 5q into p-arm of chromosome 10. There was a der(5)t(5;9;22)(q13;q34;q11.2), and der(22)t(5;22)(?;q?). Case 7 (Table: 9), apart form abnormalities observed in conventional cytogenetic method, t(12;21)(q?;q22) was observed with the help of M-FISH. Case 8 [Table: 9], the add(3)(p?) was turned out to be der(3)t(3;5)(p?q)t(5;6)(q31;?) with the help of M-FISH. There was no ?t(5;15)(q31;q25), it was der(15)t(6;15)(?;q25). add(17p) was turned to der(17)t(6;17)(?;p11) after M-FISH and der(5)t(3;5)(p?q;31) was also detected which was not possible through conventional cytogenetics. The data suggested that in each complicated case, M-FISH is required for further characterization. Zhu Y et al (2007) also reported that M-FISH can refine CCR and find out or correct the missed or misidentified abnormalities.
**Impact of BAC-FISH:**

FISH studies with commercially available probes have established the importance of FISH tests for diagnosis and therapy monitoring in CML. However, the commercial probes have limitation to reveal to understand the no. of mechanisms involved in the formation of the variants or masked Ph chromosome. Therefore, in the present series BAC-FISH was carried out in 3 patients; Category: 3, case no: 11 [Table: 8], Category: 5, case no: 2 [Table: 10] and Category no: 1, a patient with deletion of ABL/BCR on der(9).

In case no. 11 [Table: 8] the involvement of 22q13 was observed. The 22q13 region merits further study. The BAC-FISH was performed with combination of RP11-339B21 (9q34, upstream to ABL gene, spectrum green); along with 22q13.32 BACs CTA/bk299D3 and CTA/bk799F1 (downstream to BCR gene, spectrum orange). The result with CTA/bk299D3 and RP11-339B21 showed only involvement of RP11-339B21 region upstream to the ABL. Whereas results with CTA/bk299D3 with RP11-339B21 showed insertion of ABL/BCR on 22q13.32 region, hence the breakpoint was identified [Figure: 98]. RP11-339B21 (9q34) was also involved in the fusion, which consisted mainly two genes; URM1 and CERCAM. The 22q13 region includes the gene MKL1, which is involved in a specific translocation that creates a fusion with RBM15 gene (1p13). This translocation is associated with acute megakaryoblastic leukemia (Ma et al, 2001). The PDGFB, TCF20, and FBLN1 genes are also located in 22q13 region. The PDGFB (22q13.1) gene is a member of the platelet-derived growth factor family. TCF20 (22q13.31) gene encodes a protein that binds platelet-derived growth factor. FBLN1 mediates platelet adhesion via binding fibrinogen (Jamuz et al, 2010). Thus, in present case the role of these genes may play a role in leukemogenesis.

The case no: 2 [Table: 10] showed BCR/ABL on both the 9 chromosome instead of Ph. BAC-FISH was carried out using CTA/bk299D3 situated on 22q13.32 (spectrum green). Both the green signals for BAC-CTA/bk299D3, observed on both chromosomes 22, which indicated, only part of BCR gene
was inserted into 9q34 and rest of the region was remained on der(22). Thus it provided a clue for insertion mechanism [Figure: 99].

This case no: 3 showed sole Ph on karyotyping and deletion of ABL/BCR on der(9) using DCDF probe. BAC-FISH was carried out using BAC-RP11-92B2 (spectrum green) situated on 9q34 region, below the ABL gene. Both the green signals were observed on both chromosomes 9, which indicated, only part of only ABL/BCR gene was deleted on 9q34 and rest of region was remained on der(22). BAC-FISH results in this case provided a clue for interstitial deletion mechanism [Figure: 100].

Thus the present study demonstrated that BAC-FISH is an effective tool not only for mapping but also to understand the mechanisms playing role in variants. Albano et al (2007) have reported BAC/PAC FISH based on the use of clones specific for the ABL and BCR genes and for the flanking regions, which was more successful in investing genomic microdeletions on der(9) in CML and in revealing the involvement of a third chromosome in variant t(9;22) rearrangements. It is also accounted that in each case with variants further studies should be performed to confirm the presence of true breakpoint hot spots and assess their implications in CML (Virgili et al, 2008).

**Structural Changes:**

To date, more than 900 CML case with complex rearrangement have been reported (Mitelman, 2010). Any of the chromosomes can be involved in variant Ph rearrangements. The frequencies of involvement of particular chromosomes in structural rearrangement were compiled [Figure: 96]. In the present study, the most common chromosome was 17 X13. The second most involvement was seen of chromosome 1 X8. The chromosome 11 X6 and chromosome 5 X5. The chromosomes 2, 3, 6, 7 and 12 X4. The chromosome 15 X3. The chromosomes 9 and 13 X2 and chromosomes X, Y, 4, 8, 18, 21 and 22, X1. Although chromosomes were not involved in the present series were 14, 16, 19 and 20. However, they have been reported in other recent studies (Bennour et al, 2009).
At present it appears that variant translocations can affect any chromosome. However, it has been suggested that distribution of the break-points is non-random with the chromosomal bands most susceptible to breakage being: 1p36, 3p21, 5q31, 6p21, 9q22, 10q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12 and 22q13 (Bennour et al, 2009). In the present study, total 9 nonrandom breakpoints were identified; 5q13, 7q22, 10p15, 11p15, 11q13, 12q13, 13q14, 17p13 and 17q25 [Figure 97].

**Therapy outcome in relation to Glivec (Targeted Therapy)**

Total 333 patients were treated with Imatinib mesylate (Glivec), out of that 262 (79%) showed completed hematologic response, 69 (20%) patients showed no response and 2 (1%) patients were lost to follow-up. Glivec-treatment outcome in was compared between first five categories (n=294). Out of 254 patients with sole Ph showed complete hematologic response in 202 (72%) and no response in 52 (28%) of the patients. The patients with clonal evolution from first categories (n=25) and 25 patients from categories 2-5 showed complete hematologic response in 33 (66%) and no response in 17 (34%) patients.

Imatinib has been licensed since the year 2003 for use in children; however, its efficacy has so far been evaluated only in prospective trials with small patient numbers. Long-term experience in pediatric patients is still very limited and the durability of responses, as well as long-term side effects of this drug, must still be determined. In the present study out of 11 patients, 9 were given Glivec (300→400mg). 6 patients showed complete hematologic response and 3 patients showed no response. Total 4 patients showed complete cytogenetic response and 1 patient showed no response. 2 patients were lost to follow-up and 2 patients expired during the course of the treatment.

Glivec is a specifically targeted drug that inhibits the tyrosine kinase activity of BCR/ABL protein. Imatinib has impressive results in newly diagnosed patients with CML and also in previously treated CML patients in late chronic phase. In
the present study, overall survival was determined between following groups to broaden the pool of available information about the clinical relevance of additional chromosomal changes as compared to sole Ph.

In this analysis, we identified cytogenetic clonal evolution to be an independent prognostic factor for hematologic and cytogenetic response of CML patients on Imatinib mesylate therapy, even though it did not affect independently the achievement of major or complete cytogenetic response. This finding suggested clonal evolution as a single feature of CML progression. The similar results were also reported by Cortes et al (2003). However, Holzerova et al (2009) showed that Glivec treatment can be successful in patients with additional chromosomal changes. Nonetheless, their observations were based on lesser number of patients.

In nut shell, present work spurred intense evaluation of cytogenetic changes in CML. Documentation of the Philadelphia chromosome is the bonafide genetic signature of CML at diagnosis and during disease course. The molecular analysis of t(9;22) variants is needed to understand its phenotypic consequences. It has opened a way to combined molecular therapies in order to counteract the secondary oncogenic effect which is resistant to Imatinib treatment. The development of Ph-negative clones seems to be more frequent in patients treated with Imatinib than has been observed during other therapeutic regimens. Heterogeneous genetic status at diagnosis of CML may exist, underlying the need for further molecular investigations for more precise molecular disease characterization leading to better therapeutic decisions.