Prologue
A new approach to an old problem:

Cancer care and research have moved more and more into an era, in which cancer diagnostics and cancer therapies are based on the specific genetic aberrations found in the cancer to be treated. Some of the genetic aberrations serve as efficient prognostic markers in a particular disease, while others are even used as targets for therapeutic agents. Today in 2010, when genetic changes in cancer; especially hematologic malignancies are accepted as central players, it is hard to believe certain facts which paved a way for modern discoveries. Looking at the history of genetic aberrations and cancer, the idea that chromosomal alterations might be the cause of cancer was already put forward in 19th century. In 1890, the German biologist, David von Hansemann noticed that tumour cells with chromosome abnormalities also contained several spindle bodies and other mitotic aberrations, and suspected the causative role of chromosome aberrations in the initiation and progression of human neoplasia (Hanseman, 1890). Later, at the beginning of the 20th century, in 1914, the German zoologist, Theoder Boveri, proposed the paradigm that mutations in somatic cells cause uncontrolled cell proliferation. This ushered in an era of a more rational approach to chromosomal studies in oncology (Boveri, 1914). If we look 50 years back, we may realize how different the landscape was then. In 1957, knowledge of the correct chromosome number of man was 1 year old, and noble prize research that DNA is a double-helix was only 4 years old. Between 1956 and 1960, researchers identified specific chromosome abnormalities associated with congenital abnormalities (e.g., Down, Turner, and Klinefelter syndromes) and with cancer.

The advancement and importance of cancer cytogenetics were then thoroughly assessed and greatly appreciated by Dr. Janet Rowley (2001). She expressed ..."Although it has been clear for more than a century that the chromosomes in human tumour cells are often wildly abnormal, there has been controversy as to whether these changes are primary events or are merely secondary epiphenomena that reflect the genomic instability of these cells. The prevailing view for most of this period was that chromosome
changes were secondary events. What happened to change this view?” She emphasized the view that it was all technologic and certainly improvements in procedure and staining methods which have had a major impact on cytogenetics, as on other scientific endeavors.

**Chronic Myeloid Leukemia: An Historical Perspective**

Once the role of chromosomes in cancer was established, chromosomal reports related to leukemia and various myeloproliferative diseases continued to be published in the 1960s. Then the requirement of precise understanding of the molecular pathogenesis of a disease was emerged. For that, different molecular techniques allowed to know the genetic causes of leukemia. The peculiar small chromosome, a constant feature of chronic myeloid leukemia (CML), the Philadelphia (Ph) chromosome—lent further support to this idea (Nowell and Hungerford, 1960), and linked to the Boveri’s hypothesis that, "Somatic chromosomal aberrations can cause cancer". This was a very important but perplexing observation. Because it was unique, it immediately raised the question, was it a sign that chromosomal changes were important in leukemia or was it just an aberration? The consistency of the Ph chromosome was confirmed worldwide, so there was no question of its validity. Developments in banding techniques in 1969 and 1970 allowed characterization of this aberration as caused by a balanced translocation involving chromosomes 9q and 22q (Rowley, 1973).

Developments in molecular techniques allowed cloning of the affected genes by positional cloning. In 1983, Nora Heisterkamp showed that the *ABL* locus was adjacent to the breakpoint. With the help of colleagues, she cloned translocation- associated sequences from both chromosomes 9q34 (*ABL*) and 22q11 (Heisterkamp et al, 1983). In 1984, John Groffen and colleagues examined DNA samples from 19 CML patients for rearrangements on chromosome 22 using Ph translocation breakpoint probes. They found that the Ph chromosome breaks usually occurred within a 5.8-kb region, which they named the ‘breakpoint cluster region’ (*BCR*) (Groffen et al, 1984). In 1984, CML cells were shown to express an altered form of c-*ABL* with tyrosine
kinase activity, indicating a mechanism of action for this oncogene and oncoprotein (Konopka et al, 1984). Analysis of mRNA from CML patients revealed that the t(9;22) created a fusion between the 5’ part of BCR and the 3’ part of ABL (Shtivelman et al, 1985). Further, there was enormous research which identified cancer-causing fusion protein as a tyrosine kinase, and also led to the development of the kinase inhibitors [Figure: 1]. Thus, current day targeted therapies for CML: Imatinib, Dasatinib and Nilotinib (Druker, 2008) have emerged from molecular cytogenetic characterization of CML.

Figure-1: Cytogenetic and molecular characterization of CML.

The legacy of the Philadelphia chromosome:
The Philadelphia story is a scientific success story that began with the initial observation of a chromosome abnormality and its identification as a translocation. This led to the molecular analysis of the genes involved, to the functional characterization of the genes and to the detection of their altered function due to the translocation. Finally a drug that specifically targets the defective gene product [Figure: 2] was invented. Thus, translocations as well as advancement in cytogenetic techniques have been important from the biologic as well as the therapeutic point of view. Depending on one’s
perspective, the biologic discoveries could be as important as the therapeutic importance of translocations. The last two decades have seen the molecular characterization of a large number of balanced chromosomal translocations with the discovery of fusion gene and aberrant gene regulation. These efforts have provided us with the conclusive verification of Boveri’s hypothesis. Now it is well accepted that cancer is a genetic disease with two major types of initiating genetic events: (i) The inactivation of tumor suppressor genes by deletions, point mutations or may be even by epigenetic mechanisms and (ii) the activation or deregulation of oncogenes by point mutations, amplifications or balanced cytogenetic abnormalities like inversion, insertion or translocation. In addition to oncogene activation and tumor suppressor gene inactivation, a few other general principles underlying malignant transformation have also been postulated.

Figure-2: Timeline of history of the study of chromosome translocations in cancer (Source: Ref: Rowley, 2001).

The malignant transformation in general and also in leukemia is a multistep progression. Several somatic mutations are required in addition to single event [Figure: 3]. It has been postulated that for leukemic transformation at least two genetic events are required with one of this genetic lesions driving increased cellular proliferation (class I mutation) and the second mutation leading to a differentiation block (class II mutation) (Kelly and Gilliland, 2002)
and such mutations are described earlier. Over the last five decades, the different chromosomal abnormalities that are present in at least several patients (and are therefore considered to be recurring abnormalities) are included in a catalogue of cancer chromosome abnormalities (Mitelman, 2010). The largest numbers of chromosomal aberrations have been associated with haematological disorders. The karyotypes of malignant cells have provided us a wealth of information about the genetic and molecular basis of cancer. In furtherance, it is important to gain a better understanding of the full genetic and molecular effects of chromosome rearrangements.

![Figure-3: Cancer: Multistep-disease.](image)

To strengthen this fact, Dr. Brian Druker noted, "It is particularly fitting that this commitment to support of basic discoveries into the molecular pathogenesis of hematologic diseases has been translated into such a successful cancer therapy...With the tools we currently have in hand to understand disease pathogenesis, it will be truly exciting to see what will be accomplished in the next several decades"!... Using current knowledge of techniques available in cytogenetics, various chromosomal aberrations and their correlation with diagnosis, prognosis and treatment outcome in CML have been well established.
The bio-scope of the present study:

Till date, total 58,145 aberrations are reported in Mitelman Database of Chromosome Aberrations in Cancer database. Total 3527 cases are reported with CML and of which 976 cases are of aberrant CML worldwide (Mitelman, 2010). Thus, the cytogenetic profile in CML has great significance in terms of diagnosis, staging, treatment and prognosis. There are reports on CML cytogenetics from Western and European countries. However, there is a great paucity of the reports especially larger series with the recent available therapies, to document cytogenetic abnormalities in CML patients from India. Therefore, the present study was undertaken with following major aims.

1. To evaluate the clinical significance of cytogenetic studies in CML.
2. To identify the consistent sites involved in chromosomal rearrangements in CML patients.
3. To identify the rare, novel and common rearrangements and their significance in CML.

To fulfill these aims; the study objectives were to:
1. determine the frequency of Ph chromosome in CML at the diagnosis and during follow-up post-treatment.
2. determine the frequency of other chromosomal abnormalities in Ph+ CML at disease onset and during the course of treatment.
3. evaluate the frequency of simple and complex variant translocations and to find out the prognostic significance of such aberrations.
4. find out the significance of complex chromosomal rearrangement by applying (Multi-color-Fluorescence in situ hybridization) M-FISH.
5. study the molecular mechanisms involved in variant translocations with help of conventional and molecular cytogenetic techniques (FISH, M-FISH).
6. know the role and significance of place variation of BCR/ABL gene in Ph-ve CML.
7. map the adjacent chromosomal regions near the translocations’ breakpoints with the help of (Bacterial Artificial Chromosome) BAC-FISH.
8. document rare and novel aberrations in the global information pool.
9. evaluate the prognosis in terms of targeted therapy, i.e. Imatinib mesylate.