Epilogue
Over the past decade, insights into the origins and behavior of human cancers have reshaped our understanding of oncology and have brought major advances in clinical care. Using the knowledge and technologies originally derived from basic science laboratories, investigators have shifted the forefront of cancer research from in vitro models of tumor growth to the characterization and treatment of cancers in humans [Burstein HJ et al., 2008]. In AML, as a result of translocations, fusion genes are developed with novel oncogenic properties. For instance, the presence of t(8;21) translocation or a inv(16) identifies patients with a comparatively good prognosis. Whereas, the t(9;22) is associated with a poor outcome. It is important to note that chromosomal translocations have been used to identify patients who will benefit from intensifying the dose of chemotherapy. Mainly, chromosome translocations are closely associated with a particular morphologic or phenotypic subtype of AML. Cloning the genes at the breakpoints of these rearrangements had a major impact on our understanding of the molecular biology of AML. Thus, cytogenetic or molecular genetics methods have become an essential part of the routine diagnostic evaluation and follow-up of AML patients. Characterization of the functions of genes involved in these translocations has enriched our understanding of their roles in leukemogenesis, and has provided significant suggestions for newer therapies [Hayashi Y 2000].

The fascinating concept for oncogenomic data mining is the combination of the accumulated cytogenetic data with the molecular cytogenetic data from metaphase and array-based CGH experiments. The largest publicly accessible resource for molecular cytogenetic screening data in oncology is the Mitelman Database of Chromosome Aberrations in Cancer which describes more than 58145 cases till date [Mitelman 2010]. The search for recurrent breakpoint sites is important as it could help to find the localization of newer oncogenes or tumour suppressor genes. Another useful database is the ‘Atlas of genetics and Cytogenetics in oncology and Haematology’ [http://www.infobiogen.fr/services/chromcancers]. It provides information in the forms of concise and updated reviews or longer tests, a case report section, a huge portal towards genetics and/or cancer databases and
teaching items in genetics for students in medicine and the sciences. This database is made for and by clinicians and researchers in the field of cytogenetics, who are also encouraged to contribute. It deals with cancer research, genomics and cytogenetics. It is at the crossroads of research, post university teaching and telemedicine [Huret JL 2001]. In this database, the leukemia cases were divided cases into rare, novel and recurrent chromosomal abnormalities. The documentation of these chromosomal abnormalities in database is advancing the knowledge for developing newer therapies.

The present investigation is a single large series on evaluation of chromosomal abnormalities carried out from Gujarat in India, in 321 AML patients using conventional cytogenetic, FISH and M-FISH techniques. The study documented several noteworthy AML cases with rare, recurrent and novel chromosomal abnormalities. The results are briefly summarized below:

**Summary:**

**General observations:**

- 321 patients; 194 (60.4%) males and 127 (39.5%) females with age range of 1-75 years were enrolled in the study. According to FAB classification patients with different AML groups were as follows: M2 104 (32.4%), M1 95 (29.5%), M3 91 (28.3%), M4 14 (4.4%), M5 8 (2.5%), M6 8 (2.5%) and M7 only 1 (0.3%) patient.

- AML is a malignancy of male predominance.

- According to cytogenetic risk group there were 109 (33.9%) patients in favorable group, 197 (61.4%) patients in intermediate group and 15 (4.6%) patients in adverse group.

- From 321 patients, trisomy 8 was observed in 5.2% (n=17) patients. Out of 321 patients, trisomy 8 as a sole was observed in 2.1% (n=7) patients, as a secondary in 1.8% (n=6) patients and in 1.2% (n=4) patients as Complex Chromosomal Rearrangement (CCR). From all these 17 patients 2 patients
showed tetrasomy and expired, whereas 1 patient with sole trisomy 8 and 1 patient with t(15;17) were expired.

- Study also revealed several rare (n=9) and novel (n=9) case reports.
- From 321 patients, normal karyotype observed in pediatric patients and patients with younger age.
- Gain of 7p and loss of 7q chromosomal material, rather than a rearrangement of a specific gene suggest genomic imbalance associated with poor outcome.
- No hematological response was observed higher in adverse group patients.
- AML is a heterogenous disease; the treatment and outcome of patients depend on several factors, including cytogenetic abnormalities. No significant results for overall survival of paediatric and adult AML patients in terms of hematologic response.
- There was no significant difference in overall survival rate observed between three different cytogenetic risk groups; However adverse risk group patients showed very less overall survival as compared to patients with favorable and intermediate risk group.

**Favorable Risk group:**

- Recurring chromosomal abnormality; sole t(8;21)(q22;q22) was observed in 42.2% (n=45) patients.
- Sole t(15;17)(q22;q22) a recurring chromosomal abnormality; was observed in 70.6% (n=41) patients.
- The recurring chromosomal abnormality; sole inv(16)(p13;q22) were observed in 50% (n=3) in favorable risk group.
- Out of 109 patients, sole t(8;21)(q22;q22), t(15;17)(q22;q22), inv(16)(p13;q22), were observed in 58% (n=63) patients whereas, secondary changes or additional abnormalities were observed in 42.2% (n=46) patients in favorable risk group.
- Out of 109 patients in favorable risk group, loss of sex chromosome with t(8;21)(q22;q22) was observed in 1.83% (n=20) patients.
Out of 20 patients with loss of sex chromosome, the male to female ratio was 3M:1F. in these patients. Loss of Y chromosome was observed in 75% (n=15) male patients and loss of X was observed in 25% (n=5) female patients.

del(9)(q) with t(8;21) was observed in only 0.9% (n=1) patient.

Rare chromosomal abnormalities including trisomy 4, trisomy 15, i(7q10) and add(7q) were also observed with t(8;21)(q22;q22). The patient with i(7q10) was expired within a week of diagnosis which was indicator of poor prognosis.

t(1;21)(q22;q22) was found in a female patient which was a variant of t(8;21)(q22;q22).

ider(17)(q10) was found in 3 patients, 2 patients showed at diagnosis whereas 1 patient showed this anomaly at relapse and 1 female patient who was in relapsed and 1 female patient who showed this anomaly at diagnosis were expired during the course of treatment. So ider(17)(q10) might be associated with poor prognosis.

Out of 41 patients with sole t(15;17)(q22;q22), 1 patient showed disease progression and developed clonal evolution. This patient showed add(10q).

Secondary changes and additional chromosome abnormalities are related to the poor prognosis and though patient achieve complete hematological remission they had a significantly shorter survival and are twice as likely to relapse and die.

Trisomy 22 found as a secondary change with inv(16)(p13;q22) which can be regarded as an important marker for the diagnosis of inv(16)(p13;q22) in patients with AML-M4 subtype.

Rare partners of PML or RARα were observed in 2 patients in current study.

Intermediate Risk group:

In intermediate group patients, t(19;20)(p13; q22) was a novel translocation which was not previously observed. With M-FISH analysis, results revealed
that there was translocation between chr.19 and chr.20 which was difficult to identify with conventional cytogenetics.

Trisomy 13 was observed in 2 elder male patients and it was a recurrent chromosomal anomaly, and previously not observed in Indian scenario.

Sole trisomy 10 was found in 2 female patients with age of 38 and 45 years respectively.

In current study, 7 different chromosomal translocations with *MLL* gene were observed including, t(1;11)(q22;q23), t(6;11)(q;q23), t(9;11)(p;q23), found in M5 and t(10;11)(p15;q23) noted in M4. t(11;17) was found in 2 patients. T(11;19)(q23p13) was found in a paediatric patient with AML M2 diagnosis. i(11)(q23) observed in 1 patient in which FISH results showed two yellow signals of MLL break apart probe on both arms of chr.11 indicating i(11)(q10).

Sole trisomy 21 was found in 4 patients, 2 with younger age and 2 were with age of 40 and 45 years respectively.

**Adverse Risk group:**

In adverse group, the chromosome abnormalities observed are complex chromosomal rearrangement (CCR), del(5q), -7, 3q rearrangements. t(2;3)(p;q) and t(3;8) (q;q) are novel translocations which is not reported previously. These translocations were recorded 14 as novel translocations.

In present study, out of 9 patients with complex karyotype, 3 patients were elder (65 year age).

Complex karyotype has poor prognosis, it is mainly observed in elder patients.

The monosomy 7 was observed in 3 paediatric male patients.

Loss of chromosomal part was observed much more often than gain in AML with a complex aberrant karyotype.

Gain of whole chromosomes was more frequent than the loss.

M-FISH helped to find out cryptic chromosomal translocations including t(1;6), t(3;17), t(1;20), t(5;11), t(19;20), t(7;12), t(8;11) in complex karyotype and proven to be a powerful and reliable method for screening of
chromosomal aberrations, which considerably increased the accuracy of cytogenetic diagnosis.

**Conclusion:**

- The present cytogenetic study revealed a great number of non-random chromosomal abnormalities. Diagnostic cytogenetics is widely recognized as one of the most significant prognostic factors in AML. Conventional cytogenetic study reliably detects chromosomal abnormalities, and this method should not be replaced by FISH. The data suggested that it should be used as a complementary method for the detection of more subtle abnormalities. Unbalanced rearrangements leading to loss of chromosomal material are much more frequent than loss of whole chromosomes in AML.

- AML patients with a complex aberrant karyotype have a poor outcome despite intensive antileukemic treatment.

- In AML with a complex aberrant karyotype, loss of chromosomal material was observed much more often than gain. Hence, loss of tumor-suppressor genes may occur and provide a more important mechanism of leukemogenesis than activation of oncogenes. Numerical abnormalities in complex karyotype may affect gene-dosage and may play a significant role in the pathogenesis of AML.

- Secondary changes may result from genomic instability caused by the primary event. These secondary changes may provide an advantage for the transformed cell, because they are frequently found in a majority of malignant cells from a given patient. In newly diagnosed patients with APML and t(15;17), approximately 30–45% have leukemic cells with secondary abnormalities. The most common secondary event is trisomy of chromosome 8, occurring in 15–40% of t(15;17) patients, but a variety of additional chromosomal abnormalities have been also described. The high frequency of these secondary events has led to the hypothesis that *PML-RARA* expression somehow may cause genomic instability, similar to that described for the expression of *BCR-ABL* in patients with CML.
❖ This study highlights the importance of diagnostic cytogenetics as an independent prognostic factor in AML, providing the framework for a stratified treatment approach of this disease.

❖ Karyotyping and FISH both have their advantages and should ideally be carried out together. However, in serially repeated samples, karyotyping need not be repeated each time, as the change in percentage of normal and abnormal cells which is better detected by FISH is of greater importance.

❖ Cytogenetic studies should be performed at the time of diagnosis. There is increasing evidence of nonrandom chromosomal rearrangements in some of the subtypes in the French-American-British classification, which have important prognostic significance.

❖ FISH is a complementary method for the identification of inv(16) and t(11q23) in all patients with newly diagnosed AML because these abnormalities may be difficult to detect. Furthermore, molecular cytogenetics using the probe set presented in this study, provides a valuable tool for patients with poor chromosome morphology and those with a low or no yield of assessable metaphase cells.