Epilogue

The cross-hybridization of cytogenetics and molecular genetics has led to conceptually new advances and insights into the fundamental cell biology mechanisms that are disrupted when neoplastic transformation occurs. The clinical usefulness of cytogenetic findings as diagnostic and prognostic aids in cancer medicine has been increasingly appreciated which has great advantage to clinicians and researchers. Cytogenetics has become a central methodology in basic and clinical cancer research, pharmacogenomics, proteomics and an important clinical tool in oncology. It is known that wealth of cancer karyotype merits additional investigations, which provide more insights on the role and importance of cytogenetics in leukemia.

In online Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, 8871 cases are reported with ALL worldwide [Mitelman F. et al; 2013]. There are very few reports from India and only in form of either case reports or smaller series on ALL cytogenetic. However, aforementioned reports on ALL cytogenetics are mainly from Western and European countries.

The present cytogenetic exploration is a single large series on assessment of chromosomal abnormalities carried out from Gujarat in India, in 273 ALL patients using conventional cytogenetic, FISH and M-FISH techniques. The study documented several noteworthy ALL cases with rare, recurrent and novel chromosomal abnormalities.

The results of the present study are summarized below.

❖ General Summary:

➢ Total 273 ALL patients were characterized for karyotyping.
➢ ALL is a malignancy of male predominance.
➢ Among 273 patients 70.7% males and 29.3% were females, M:F ratio was 2.4:1.
➢ Among all 273 patients; 3% were infants, 52% were children and 45% were adults.
➢ There were total 5 novel and 11 rare cases observed in the present study. It suggested that chromosomal regions involved in novel and rare rearrangement may provide clues for identification of genes underlying leukemogenesis.
Comparative studies were carried out for different parameters according to (1) cytogenetic categories (2) cytogenetic risk groups according to WHO classification (3) FAB classification and (4) Immunophenotype (IPT) sub groups.

Distribution of patients according to cytogenetic category

All 273 patients were divided into six cytogenetic categories according to cytogenetic abnormalities; i.e. 1. t(9;22)(q34;q11.2) (n=27) 2. t(9;22)(q34;q11.2) with hyperdiploidy (n=10) 3. hyperdiploidy (n=26) 4. hypodiploidy (n=20) 5. normal karyotype (n=149) and 6. miscellaneous group (n=41).

Category 1. t(9;22)(q34;q11.2) (27 patients)
- Maximum female patients (51.9%) as compared to male patients (48.1%) were observed in t(9;22) category.
- Maximum adult patients (81.5%) as compared to children patients (18.5%) were observed in t(9;22) category.

Category 2. t(9;22)(q34;q11.2) with hyperdiploidy (10 patients)
- Patients having t(9;22) with hyperdiploidy were older (p<0.0001) with mean age 37.4 years among the 6 evaluated cytogenetic categories.
- Only adult patients were observed in t(9;22) with hyperdiploidy category.

Category 3. Hyperdiploidy (26 patients)
- Maximum male patients (92.3%) as compared to female patients (7.7%) were observed in hyperdiploid (2n+) category.
- Patients with hyperdiploidy represented the most frequent group 34.8% (16/46) and more common 24% (11/46) in patients with age group of 1-15 years.

Category 4. Hypodiploidy (20 patients)
- Maximum male patients (70%) as compared to female patients (30%) were observed in hypodiploid (2n-) category.
- Maximum patients (45%) observed with 45 chromosomes in hypodiploid (2n-) category.

Category 5. Normal karyotype (149 patients)
- Maximum male patients (74.5%) as compared to female patients (25.5%) were observed in normal karyotype category.
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- Maximum children patients (59.7%) as compared to adult patients (36.9%) and infant patients (3.4%) were observed normal karyotype category.

Category 6. Miscellaneous category (41 patients)
- Cytogenetic abnormalities other than t(9;22), hyperdiploid, and hypodiploid are included in miscellaneous category.
- The chromosomal abnormalities included were: add(1)(p36), r(2), del(2)(p23), del(6q)(q21), add(7)(q34), del(8)(q22), del(9)(q22), del(11)(q23), add(13)(p11), add(14)(q11), add(16)(q24), del(16)(q22), add(17)(q?), del(19)(?p), del(20)(q11), t(1;4;6;11)(q31;q27;q22;q23), t(1;19)(q21;p13), t(1;22)(p13;q13), 2;11)(p16;p15), t(4;11)(q21;q23), t(5;7)(?q;?q), t(5;13)(q31;q34), t(6;11)(?p;?q), t(6;12)(q21-22;p13), t(6;19)(q16;p13), t(8;12)(q21.1;p12), dic(9;12)(p13;p12), t(11;14)(p14;q21), t(11;15)(p15;q22), Complex chromosomal rearrangement.
- The WBC counts were significantly higher (P<0.019) in patients with miscellaneous group (mean WBC count $8.65 \times 10^3$ /cm^3) among all the 6 evaluated cytogenetic categories.

Overall survival within 6 cytogenetic categories:
1. The overall survival was evaluated in six cytogenetic categories with 273 ALL patients indicated that the patients with hyperdiploidy (23.49% OS, CI, 13.65-33.33) confers good prognosis. Patients with the presence of Philadelphia chromosome (1.40% OS, CI, 0.47-2.32) confers an adverse prognosis (p<0.0001).
2. Pair-wise comparisons of OS in t(9;22) category (1), t(9;22) with hyperdiploid category (2) and hyperdiploid category (3) were carried out. The survival was shorter in ALL patients with t(9;22) with hyperdiploidy as compared to the patients with t(9;22) category. Thus, it is believable that the simultaneous presence of additional karyotypic abnormalities may alter the biological properties of Ph positive cells and influence clinical outcome.
3. The present study indicated that DFS was higher in normal karyotype category (27.65%, CI: 23.42-31.88) and shorter in hypodiploidy category.
(15.07%, CI: 10.02-20.14) among the 188 evaluated patients of all cytogenetic categories.

❖ Numerical chromosomal abnormalities:
- Among 273 ALL patients, 17% (46) patients were having numerical chromosomal abnormalities.
- Hyperdiploidy 24% (11/46) were much more frequent among children.
- No infant patient was found to have numerical chromosomal abnormalities.
- The trisomy of chromosome 8 was the most frequent numerical chromosomal abnormality observed in 9 patients in the present study.
- The other trisomies involved gain of chromosome 5 and 21 in 3 patients. The gain of chromosome 6 and 12 were observed in 2 patients. Chromosome 4, 7, 11, 16, 17 and 18 were reported for once.
- Hypodiploidy was observed in 20 patients.
- Monosomy of chromosome 9 and 21 were observed in 2 patients. Monosomy of chromosome 15 in 1 patient and monosomy of chromosome 22 observed in 1 patient.

❖ Structural chromosomal abnormalities:
- In structural chromosomal abnormalities t(9;22)(q34;q11.2) was the most frequent (37/78, 47.4%).
- BCR-ABL variant positive was observed in 6 patients. Out of 6 patients, 3 patients observed with double Philadelphia chromosome, 2 patients with mix clone population and one patient observed with deletion of 5'ABL gene on der(9) chromosome.
- The second most common reported abnormalities were; add(1)(p36) for 5 times, t(1;19)(q21;p13) for 3 times. t(4;11)(q21;q23), del(11)(q23), del(6)(q21), and del(16)(q22) were observed 2 times. The del(9)(q22), del(19)(p?), del(22)(q?), add(7)(q34), add(17)(q?), add(19)(p13), add(X)(q?), del(2)(p23), del(1)(p32), dic(9;12)(p13;p12) and t(11;14)(p14;q21) observed once.
- The present study showed that chromosome 22 was most commonly involved chromosome (40/78, 51.3%) in structural chromosomal abnormalities. The involvement of chromosome 18 was not found in any of the patient.
The second most common chromosome involved 9 for 39 times. Other chromosomes involved likewise, chromosome 1 for 12 times, chromosome 11 for 8 times, chromosome 6 and chromosome 19 for 7 times and chromosome 8 for 6 times. Chromosome 10, 14, 15 and chromosome Y was involved only for once. Chromosomes X was reported twice in the present study.

There were 2 cases with a ring chromosome. r(2) and r(9) observed in present study, in both cases shorter survival and poor prognosis was observed.

WCP-FISH and M-FISH helped to characterize the complex chromosomal rearrangements that could not be fully defined by conventional cytogenetics.

The combined approach for identifying all the chromosomal rearrangements in a tumor cells using conventional cytogenetics and FISH (using LSI and WCP probes) was found to be more meaningful.

Distribution of patients according to cytogenetic risk groups

According to WHO classification, 273 patients were divided in to 3 cytogenetic risk groups: 1. Favorable risk group (n=186) 2. Intermediate risk group (n=8) and 3. Adverse risk group (n=79).

1. Favorable risk group
- The chromosomal abnormalities included were; hyperdiploidy, normal karyotype, add(1)(p36), add(13)(p11), del(1)(p32), del(8)(q22), t(1;19)(q21;p13) and dic(9;12)(p13;p12).

2. Intermediate risk group
- The chromosomal abnormalities included were; add(9)(?p), add(14)(q11), add(19)(p13), del(6)(q16), del(9)(q22), del(19)(?p), t(2;11)(p16;p15), t(6;19)(q16;p13), and t(8;12)(q21;1;p12).
- WBC counts were found significantly higher (p<0.001) in patients with intermediate risk group (mean WBC count 1.07x10^5/cmm) among the three cytogenetic risk group patients.

3. Adverse risk group
- The chromosomal abnormalities included were; hypodiploidy, del(2)(?p), add(7)(q34), del(11)(q23), del(16)(q22), del(20)(q11), i(17)(q10), t(1;4;6;11)(q31;q27;q22;q23), t(1;22)(p13;q13), r(2), t(6;12)(q21-22;p13),
t(9;22)(q34;q11.2), t(4;11)(q21;q23), t(5;13)(q31;q34), t(11;15)(p15;q22) and complex chromosomal abnormalities.

- Age was significantly higher (p<0.0001) in adverse group patients (mean age 24.4 years) among cytogenetic risk groups.
- In the present study, OS in patients within three cytogenetic risk groups indicated that the survival was shorter in patients with adverse risk group (14.04%, CI: 10.07-18.01) and higher in favorable risk group (20.32%, CI: 17.19-23.45) (p<0.041).
- Hemoglobin levels were significantly higher (p<0.012) in adverse group patients (mean Hemoglobin count 8.25g/dl).

### Distribution of patients according to FAB classification

Patients were divided into 4 different subgroups according to FAB classification i.e. 1. ALL-L1 (n=38), 2. ALL-L2 (n=26), 3. ALL-L3 (n=3) and 4. ALL (not classified) (n=206).

#### 1. ALL-L1
- The chromosomal abnormalities included were; add(1)(p36), del(11)(q23), t(1;19)(p13), t(1;22)(p13;q13), t(4;11)(q21;q23), t(6;12)(q21-22;p13) dic(9;12)(p13;p12), t(9;22)(q34;q11.2), +8, +21 and -21.

#### 2. ALL-L2
- The chromosomal abnormalities included were; add(1)(p36), add(7)(q34), add(17)(q22), del(16)(q22), del(20)(q11), t(1;4;6;11)(q31;q27;q22;q23), t(9;22)(q34;q11.2), t(11;15)(p15;q22), r(2) and +12.

#### 3. ALL-L3
- The chromosomal abnormalities included were; add(13)(p11) and +8.

#### 4. ALL (not classified)
- The chromosomal abnormalities included were; add(1)(p36), add(14)(q11), add(16)(?q), del(6)(q16), del(8)(q22), del(9)(q22), del(11)(q23), t(1;19)(q23;p13), t(2;11)(p16;p15), t(5;7)(q2;?q?), t(5;13)(q31;q34), t(6;19)(q16;p13), t(8;12)(q21.1;p12), t(9;22)(q34;q11.2), r(9)(q34q34), i(17)(q10), +4, +5, +6, +8, +11, +12, +16, +17, +18, +21, -X, -6, -9, -21 and -22.
- Patients having ALL (not classified) were older (p<0.0001, mean age 18.9 years) among the four evaluated FAB categories.
In the present study, median age of patients in ALL-L3 was 17 years and the estimate OS was 8.67%. However, in patients with ALL-L1 group median age was 8 years and OS was 23.19%, thus reflecting the survival disadvantage that OS continuously decreases with increasing age.

Distribution of patients according to Immunophenotype (IPT) sub-groups

Patients were divided into 2 different subgroups according to Immunophenotype classification i.e. B-cell ALL (63 patients) and T-cell ALL (15 patients).

**B-cell ALL:**
- The chromosomal abnormalities included were; \(\text{add}(1)(p36), \text{add}(7)(q34), \text{del}(20)(q11), t(1;4;6;11)(q31;q27;q22;q23), t(1;19)(q21;p13), t(6;19)(q16;p13), r(9)(q34q34), t(9;22)(q34;q11.2), +5, +6, +8\) and \(-21\).
- B-cell malignancy was more prevalent than T-cell malignancy.

**T-cell ALL:**
- The chromosomal abnormalities included were; \(\text{add}(1)(p36), \text{del}(6)(q16), r(2), t(9;22)(q34;q11.2), +4, +12, +21\) and \(-6\).
- Hemoglobin levels were significantly higher \((p<0.023)\) in patients with T-cell ALL (mean hemoglobin count 9.07 g/dl) as compared to the patients with B-cell ALL. Present study also confirmed the close association between T-cell phenotype with male gender \((60\%)\), higher WBC count \((100.5\times10^3/cmm)\), higher platelet count \((85.6\times10^3/cmm)\) and high hemoglobin level.

In the present study, OS in patients with Immunophenotype groups indicated that the survival was shorter in patients with T-cell ALL \((10.73\%, \text{CI}: 5.61-15.85)\) as compared to the patients with B-cell ALL \((16.36\%, \text{CI}: 11.69-21.03)\) group.

Treatment outcome
- All 273 patients received standard conventional chemotherapy i.e. protocol MCP841. In case of Philadelphia positive patients, in addition to protocol MCP841 Imatinib Mesylate was given.
- The present study showed that 69% \((188)\) achieved complete remission (CR) and 31% \((85)\) patients were not responder to therapy among the 273 ALL patients.
- Present study confirmed that a Philadelphia chromosome remains a major prognostic factor of induction failure reported in 49% \((18/37)\) patients,
resistance to treatment and associated with high rates of relapse reported in 22% (8/37) patients. Total 71% (26/37) patients showed poor prognosis.

Among the different cytogenetic categories, not a single patient with hyperdiploidy and t(9;22) reached to maintenance cycle. Whereas, maximum patients i.e. 62% (92/149) in normal karyotype category, received maintenance cycle.

**Conclusion**

- Karyotyping is a powerful technique that provides a global picture of the entire genetic constitution of a cell.
- Performing classic cytogenetics both at diagnosis and during the course of the disease is still the only way for characterizing the ALL patients. As the presence and/or the development of additional chromosome changes before and during Imatinib therapy suggests important pathogenetic, prognostic and, consequently, therapeutic implications. The combination conventional and molecular cytogenetic analysis can bring to light the frequency of cytogenetic alterations, identifying prognostic value in correlation with prognosis. These technologies result in systematic characterization of cytogenetic alterations, allowing investigators to identify the underlying genetic changes found in ALL.
- Any of the chromosomes can be involved in structural and numerical abnormalities and may exhibit non-random breakpoints.
- The CCR indicates genomic instability; chromosomal breakpoints can unravel genomic regions important in disease progression. The analysis of CCR by M-FISH or by similar sensitive molecular methods is important.
- The study documents that such examinations of large cohorts of ALL patients could lead to detection of new or nonrandom complex rearrangements. These rearrangements play a significant role during progression of malignant disease.
- The data suggested that cytogenetic is the most important factor that predicts treatment outcome in ALL. A detailed risk stratification based on cytogenetic and molecular abnormalities could also be established for ALL patients. Hence, new clinical trials targeting with different age group and specific cytogenetic abnormalities are urgently required.
The strength of this study is novel and rare cases observed in this cohort.

The study demonstrated crucial role of FISH and usefulness of M-FISH analysis in disclosing CCR.

The combined approach using CC and FISH can bring to light the frequency of hidden cytogenetic abnormalities. Correlation of these abnormalities with prognosis is an important component in assessing the risk stratification of ALL. Such an approach may identify patients who could benefit from newer therapeutic approaches.

The results reported here demonstrated that the scenario of ALL may differ from developed nation.

These intriguing data suggests that further international surveys of molecular epidemiology of ALL, matched with ethnic and socioeconomic information, may provide new insights into leukemogenesis in both developing and developed countries.

To improve the survival of patients with ALL in developing countries, it is important to conduct research into the biology, response to treatment and prognostic factors of the disease in the developing countries themselves.

Furthermore, CC analysis may well lead to the continuous discovery of new, relevant genetic events in leukemia, despite our nascent molecular centricity as this has significant value in overall survival and in prognostication.

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 ALL. 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 ALL.

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

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 future, it would be very useful and essential for the advanced cytogenetic centers like ours' and other cytogenetic laboratories to routinize quantitative PCR, playing key role in diagnosis and management of patients with ALL and other hematologic malignancy. DNA-sequencing to rule out kinase domain mutation analysis in patients with Imatinib resistance will be essential. Micro-Array for mapping of nonrandom breakpoints involved in complex chromosomal rearrangements will also be needed.