SUMMARY

The work presented in this thesis is an attempt to characterize structural and functional changes of some relevant genes in leukemia patients in India. The work can be sectioned into four parts. The first part deals with change in the expression of some tumor suppressor genes and oncogenes in acute myeloid leukemia (AML). The second part deals with analysis of the promoter methylation status in some tumor related genes. The third part is an attempt to characterization of common chromosomal translocation and gene alteration in AML and chronic myeloid leukemia (CML) patients. The last part is the study on genetic polymorphisms on CML patients with respect to normal population.

Expression levels of seven cell cycle related genes, p16INK4A, p14ARF, p15INK4B, p21CIP1, p53, MDM2 and Bcl-2 in bone marrow/ blood samples from 61 de novo AML patients and 14 controls from eastern India was determined using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) method. Increased mRNA expression of MDM2 oncogene (P = 0.018) and p14ARF, lower expression of p21CIP1 was detected in patients' samples. Patients with high expression of MDM2 had considerably shorter overall survival relative to low expressed group (P = 0.03).

The expression patterns of p73 C-terminal isoforms, ΔNp73, and exon 2 polymorphisms of p73 gene and allelic expression of p73 in de novo AML samples have been investigated. Differential expression of p73 isoforms has been identified all in AML subtypes and ΔNp73 is expressed in 32% of patients. No association was found between survival of the patients with ΔNp73 expression and exon 2 polymorphisms. However, lack of p73 α, β, and ε isoforms expression relate to good prognosis.

Aberrant methylation of promoter region CpG islands of nine cancer related genes, i.e. p15, p16, p14, p73, p21, E-cadherin, hMLH1, retinoic acid receptor β2 (RARβ2), and suppressor of cytokine signaling-1 (SOCS1) have been analyzed by methylation specific polymerase chain reaction (MSP) of bisulfite treated DNA from 63 de novo AML patients. Aberrant promoter methylation was detected in 80% (50/63) of the patients belonging to all French-American-British (FAB) subtypes. The frequency of aberrant methylation was 39.7% (25/63) for RARβ2, 27% (17/63) for E-cadherin and hMLH1, 23.8% (15/63) for p15, 22.2% (14/63) for p16, 15.8% (10/63) for p14, 14.3% (9/63) for p73, 20% (8/40) for SOCS1 and 0% (0/63) for p21. Survival of the patients decreased significantly (P = 0.015) with increase in number of genes hypermethylated in a patient.

In the third part, the FLT3-ITD and FLT3-D835 mutations status have been detected in 63 de novo AML patient samples at diagnosis and explored possible correlation between FLT3 alteration and clinical parameters; and also have
investigated 54 AML patients (excluding AML-M3 subtype) by RT-PCR in order to establish the frequency of AML-ETO rearrangement in AML of different subtypes of the FAB classification. This study indicates that FLT3-ITD mutation (11%) and AML1-ETO transcript in AML-M2 subtype were detected less frequently (19.5%) than reported in other studies.

The distribution of BCR-ABL transcript type was studied by RT-PCR in CML patients, which is probably the first such report on Indian population is consistent with the studies on Caucasian population (b2a2: b3a2:: 40:60) and not with Ecuadorian population (b2a2: b3a2:: 95:5). b2a2 transcript carrying patients were relatively younger with higher WBC counts. No correlation between transcript type and platelet counts at diagnosis was found. Phenotypes of two e19a2 cases identified were of typical CML in accelerated and chronic phase which do not support the hypothesis that the transcript is associated with CML-N type. Lack of the exon 13 polymorphism in some patients of this study coexpressing b2a2 and b3a2 transcripts shows conclusively that the polymorphism does not have any role in dual expression of b2a2 and b3a2 transcripts and the intron 13 polymorphism at the putative branchpoint is necessary and sufficient for dual expression.

The association of Glutathione S-transferase (GST) gene polymorphisms with the incidence of CML was investigated. GSTT1 null genotype was detected as statistically significant risk factor of CML and is the first report of association between incidence of chronic myeloid leukemia and GSTT1 null genotype.