CHAPTER 8

SUMMARY, CONCLUSION AND FUTURE SCOPE

8.1 SUMMARY OF THE WORK DONE

Clonal TCRG and TCRD gene rearrangements were detected in 53 cases of T-ALL. Clonal gene rearrangements were detected using PCR and Homo-heteroduplex analysis. Homoduplex or heteroduplex PCR product was excised from the gel and junctional region sequences were identified in 15 cases of TCRG and 10 cases of TCRD rearrangements by sequencing. To assess the prognosis of patients showing TCRγδ+ genotype at diagnosis with patient’s response to treatment by MCP 841 protocol, the clinical outcome was compared between TCRγδ+ and TCRγδ− T-ALL cases. Kaplan-Meier method was used to estimate the EFS of TCRγδ+ and TCRγδ− T-ALL and differences compared by log-rank test.

In precursor-B-ALL, cross-lineage TCRG and TCRD gene rearrangements were detected in 71 cases and Ig heavy chain (FR1-JH) gene configuration was studied in 50 cases. Sequencing was done in 20 clonal IgH (VH-DH-JH) rearrangements to characterize the DH, JH gene segments and CDR3 sequences.

A preliminary study on Quantitation of MRD during treatment was done in 10 ALL cases (T-ALL= 6, precursor-B-ALL= 4) with 44 follow up samples using
Real-time PCR. Bone marrow DNA from presentation and follow up samples were quantitated using the standard curve generated with RNaseP gene to correct for the quantity and quality of DNA. To determine the sensitivity of the PCR target, diagnostic DNA with 90-95% tumor cell involvement was serially diluted (50 ng to 5 pg leukemic cells) in 500ng of polyclonal control DNA that is equivalent to about \(10^5\) cells that gives a final concentration of \(10^{-1}\), \(10^{-2}\), \(10^{-3}\), \(10^{-4}\), and \(10^{-5}\). The dilution series of diagnostic DNA was subjected to RQ-PCR analysis together with 500ng follow-up samples and negative control. The quantity of tumor cells in diagnostic DNA is set at 1.0 and the MRD quantities in follow-up samples were divided by the amplifiable quantity of DNA to obtain the normalized target value. For quantitation of MRD, TCRG clonal marker was used in 6 cases (T-ALL=4 and B-ALL= 2), TCRD was used in 2 cases and IgH was used in 2 cases.

Fusion gene transcripts \(mBCR-ABL\), \(E2A-PBX1\), \(TEL-AML1\) and \(MLL-AF4\) were studied in 61 cases of precursor-B-ALL by RT-PCR. Sequencing was done in samples that showed the fusion gene transcripts to check for the presence of any variants. The fusion gene transcripts \(mBCR-ABL\), \(E2A-PBX1\) and \(TEL-AML1\) were also studied and assessed by Real-time PCR based dissociation curve analysis in 61 cases of precursor-B-ALL. Tal-1 deletion Type 1 and Tal-1 deletion Type 2 were studied in 45 cases of T-ALL by DNA-PCR.
8.2 CONCLUSION

Biology of ALL in South Indian population differs from Western countries in terms of high incidence of T-ALL, paucity of c-ALL and paucity of TEL-AML1 fusion gene. In South Indian T-ALL cases, TCRG gene rearrangements were detected in 68% and TCRD gene rearrangements in 34% of cases. Though the incidence of T-ALL is more in India, it is evident from our study that the frequency of TCRG rearrangements in South Indian population is less and is different form that reported in other geographic populations. The survival rate was more for cases showing TCRγδ+ T-ALL than TCRγδ- T-ALL cases. Average size of the junctional region in TCRG was 7.4 nucleotides (range 2 to 18 nucleotides) and complete-TCRD was 27 nucleotides (range 14 to 42 nucleotides). In the present study, SmCD3 positive cases are significantly associated with absence of complete-TCRD rearrangements. In south Indian T-ALL patients, the TCRG rearrangements are more frequent than TCRD. Although the junctional region in TCRD is more diverse than TCRG, of the 53 T-ALL cases, only 10 (18.8%) cases showed at least one complete-TCRD, whereas TCRG rearrangements were detected in 36 (68%) cases. So, we rely more on TCRG clonal markers to quantitate the MRD in South Indian T-ALL patients.

In South Indian precursor-B-ALL cases, clonal TCRG gene rearrangements were detected in 47.8% cases and incomplete TCRD gene rearrangements were detected in 44% cases. In pediatric precursor B-ALL cases, the frequency of both TCRD and TCRG rearrangement pattern is slightly
less than the frequency reported from other populations. The frequency of V_{γ11} (V_{γ9})-J_{γ1.3}/2.3 was significantly more in adult precursor-B-ALL compared to pediatric precursor-B-ALL. In our study of B-ALL cases, there was no association between TCRG or TCRD gene rearrangements and clinical outcome of the patients.

IgH gene rearrangement was detected in 41 of 50 (82%) cases. The CDR3 (N-D_H-N) sequence ranged from 5 bp to 73 bp (average 28 bp). In 11 of 20 (55%) IgH rearranged sequences, the CDR3 sequence was in frame but in 9 (45%) rearrangements it was found out of frame. The present study has not revealed any significant difference in the pattern of IgH rearrangement between childhood and adult precursor-B-ALL. Also, there is no significant difference in the clinical features between patients showing one clonal IgH marker and more than one clonal IgH marker.

RQ-PCR results in 4 cases reached a maximal sensitivity of detecting one leukemic cell in 10^5 normal cells and in 4 more cases a maximal sensitivity of detecting 1 in 10^4 was reached. In this study, MRD value at maintenance phases revealed MRD-negative in five cases and showed MRD-positive in four cases. Our preliminary study shows that MRD level after treatment has no correlation with age, WBC count, blasts and immunophenotype at diagnosis. Due to the limited number of cases with different time-points of follow-up it is difficult to risk-stratify the patients. Hence, a larger number of cases with longer follow-up is needed to risk-stratify the patients based on the quantity of MRD.
Fusion gene transcripts were detected in 11/61 (18%) cases. Fusion gene transcripts \textit{E2A-PBX1} and \textit{TEL-AML1} were detected in 4/61 (6.5%) cases. \textit{mBCR-ABL} was detected in 2 (3.2%) cases and \textit{MLL-AF4} in one case (1.6%) of a one-year-old infant. The present study reiterates that \textit{TEL-AML1} fusion gene is less (6.5%) in South Indian ALL patients compared to the frequency reported (16%-29%) in worldwide. Tal-1 deletion Type 1 was detected in 6 (13.3\%) cases but tal-1 deletion type 2 was not detected in this series of cases.
8.3 FUTURE SCOPE

1. In the present study, only six cases of T-ALL have shown TCR\(\gamma^\delta^+\) genotype and the survival rate was more for cases showing TCR\(\gamma^\delta^+\) than TCR\(\gamma^\delta^-\) ve cases but it was not statistically significant due to the small number of cases of TCR\(\gamma^\delta^+\). Hence, a large number of cases with longer follow-up is needed to assess the prognostic significance of TCR\(\gamma^\delta^+\) T-ALL.

2. To target the fusion gene transcripts E2A-PBX1 and TEL-AML1 in cell lines harboring these fusion genes using Small interfering RNA (SiRNA) complementary to the fusion region and study its therapeutic impact.

3. E2A gene plays a fundamental role in regulating lymphoid development. Our study also confirms the finding that in cases showing E2A-PBX1 fusion gene, TCRG is not rearranged. Hence, in cases showing E2A-PBX1 fusion gene what happens to TCRG locus is to be studied.

4. To do a prospective study on quantitation of MRD in ALL patients during and after treatment. In addition will need to study the MRD in more number of cases with longer follow-up to enable risk-stratification of ALL patients.