T Cell Receptor Gamma and Delta Gene Rearrangements in T-cell Acute Lymphoblastic Leukemia in South India and Quantitation of Minimal Residual Disease

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Objective of the Study

- To detect the T cell receptor Gamma and Delta gene rearrangements in T-cell Acute lymphoblastic Leukemia patients in South India

- To Quantitate the Minimal Residual Disease (MRD) in follow-up samples of ALL using Real-time PCR

TCR Gene Rearrangements

- During early T cell differentiation, the germline encoded V, D and J gene segments of TCR gene complex rearrange
- Each lymphocyte gets a unique V-(D)-J segment that codes for the variable domain of TCR molecules
- Combinatorial diversity: By the number of possible combinations of V-(D)-J segments
- Junctional diversity: By the random insertion and deletion of nucleotides at the junction sites of V-(D)-J segments. The junctional regions are unique “fingerprint like sequences” different in each lymphoid clone

Patients and Methods

Patients

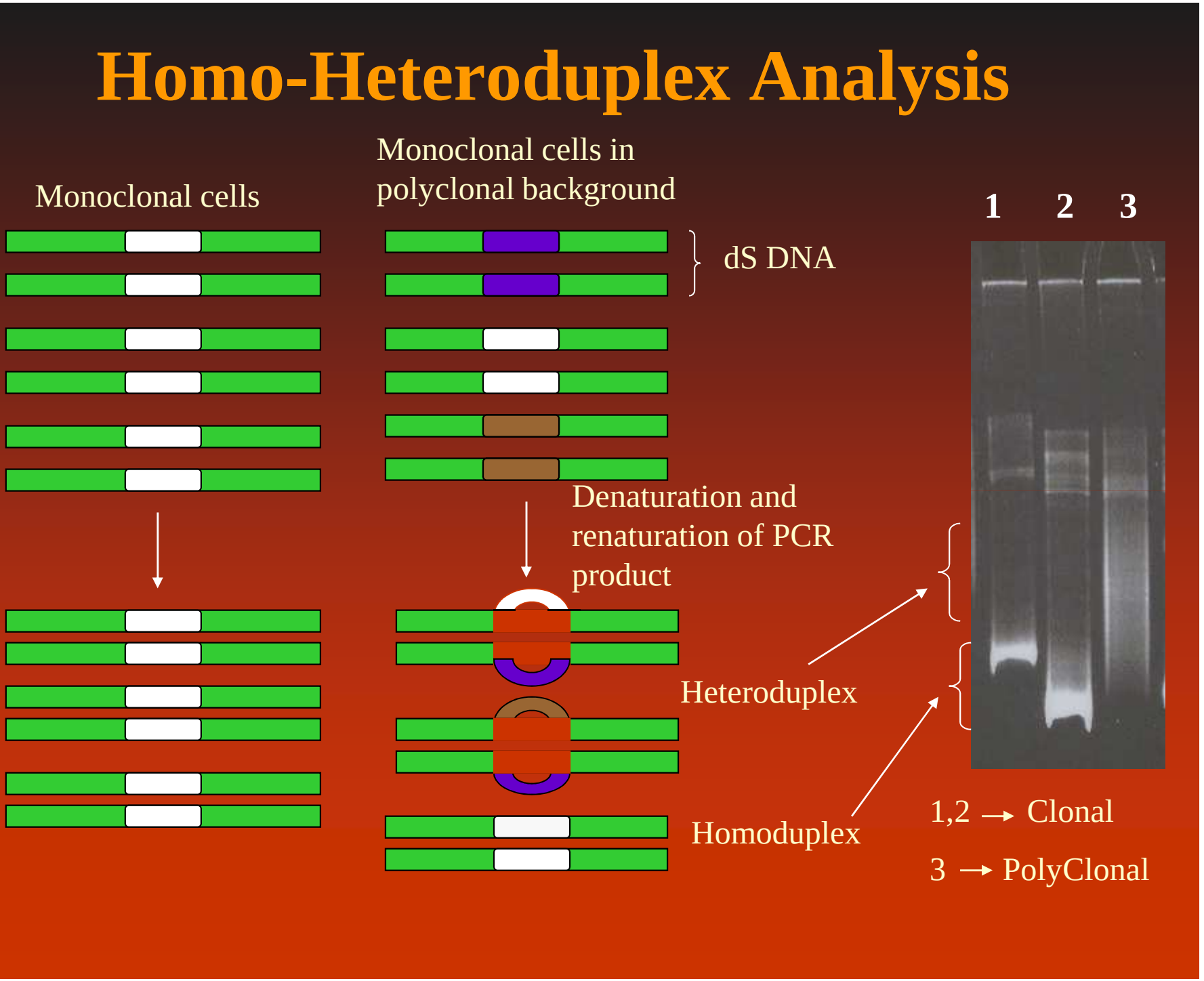
- 54 T-ALL Patients enrolled for MCP 841treatment protocol included for the study

Methodology

10 ml of PB and 2 ml BM collected from the patients were used for study

- Isolation of DNA from mononuclear cells
- PCR
- Heteroduplex analysis
- Sequencing the Homoduplex PCR product to design Allele Specific Oligonucleotide primers (ASO)
- Real-time quantitative PCR

Homo-Heteroduplex Analysis



TCRG gene rearrangements in T-ALL

- *TCRG* rearranged in 37 of 54 T-ALL cases (68.5%)
- VγI-Jγ1.3/2.3 more commonly rearranged in 29 cases (54%)
- VγII-Jγ1.3/2.3 rearranged in 29 cases (26%)
- VγIII-Jγ1.3/2.3 and VγIV-JγI.3/2.3 in 4 cases (7.4%)
- VγI-Jγ1.1/2.1 in 3 cases (5.5%)
- Junctional region sequence of *TCRG* ranged from 1 nucleotide to 11 nucleotides (average 7.6 nucleotides)

- [Ref: Sudhakar et al, American Journal of Hematology 82: 215-221 (2007)]

TCRD gene rearrangements in T-ALL

- *TCRD* rearranged in 16 of 54 cases (29.6%)
- Vδ1-Jδ1 rearranged in 9 cases (16.6%)
- Vδ2-Jδ1 and Vδ3-Jδ1 rearranged in one case each (1.8%)
- Vδ2-Dδ3 in 5 cases (9.25%) and Dδ2-Dδ3 in 4 cases (7.4%)
- Junctional region sequence of *TCRD (*Vδ1-Jδ1, Vδ2-Jδ1 and Vδ3-Jδ1) ranged from 14 to 42 nucleotides (average 27 nucleotides)

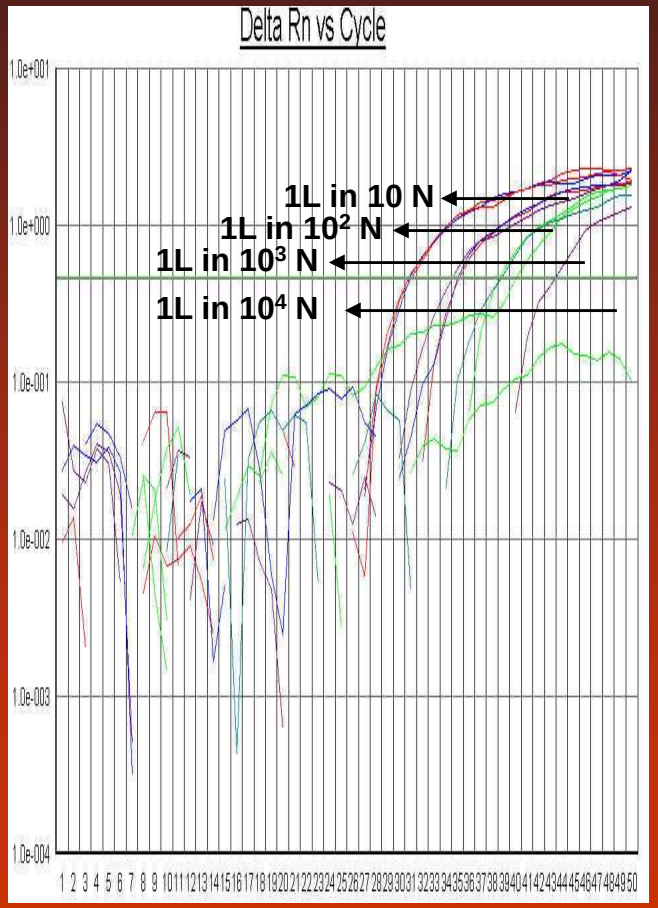
- [Ref: Sudhakar et al, American Journal of Hematology 82: 215-221 (2007)]

Real-time Quantitative PCR

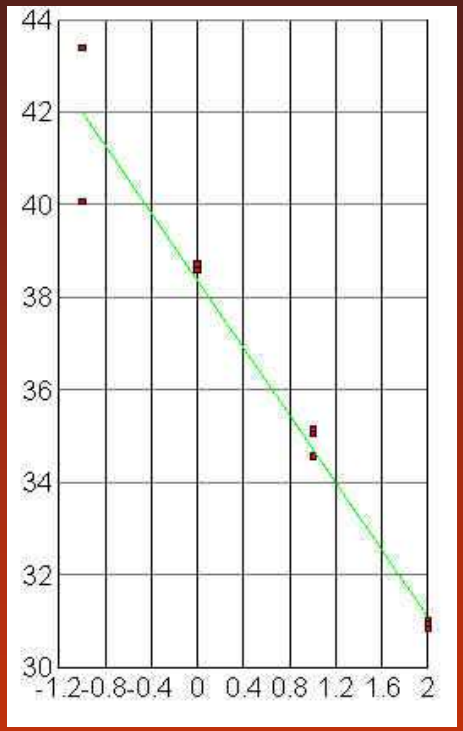
- Quantitation of DNA using a control gene (RNAse P gene)
- Quantitation of MRD
  - Diagnosis DNA with almost 100% tumor cell involvement was serially diluted (50 ng to 5 ng leukemic cells) in 500ng of polyclonal control DNA (10^5 cells) to give final concentrations of 10^{-1} to10^{-5}
  - Serially diluted diagnosis DNA (duplicates) subjected to ASO-PCR together with follow-up samples (500ng in triplicates) and negative control
  - MRD quantities divided by amplifiable DNA.

Amplification plot and Standard curve

Amplification plot

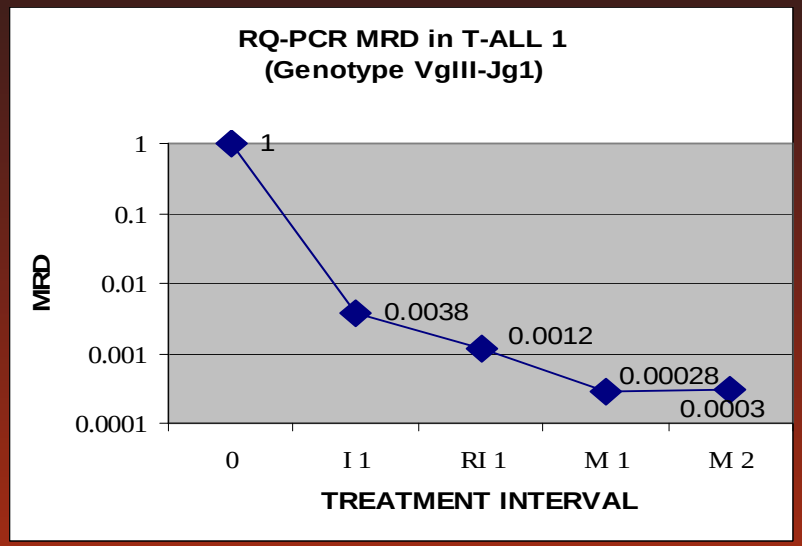


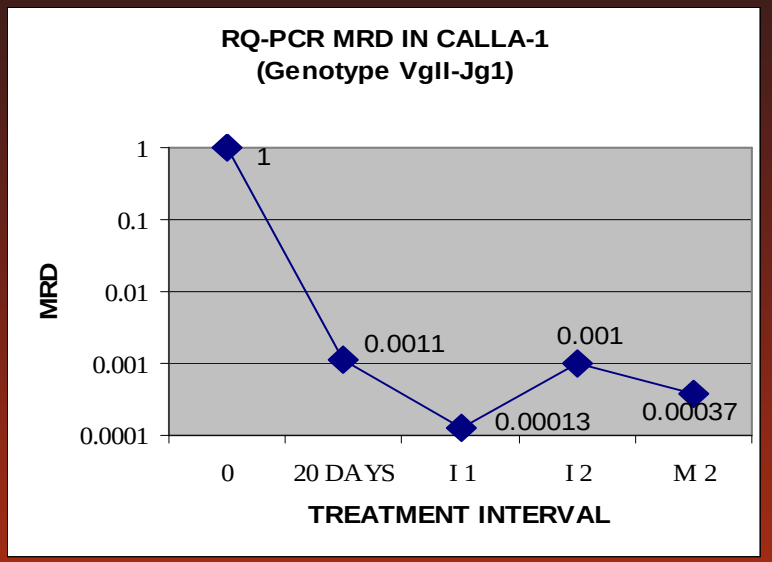
Standard curve

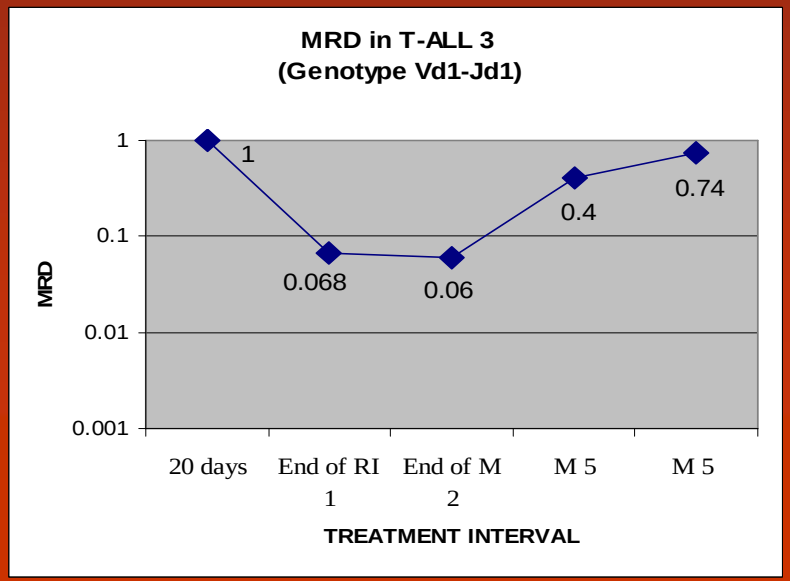


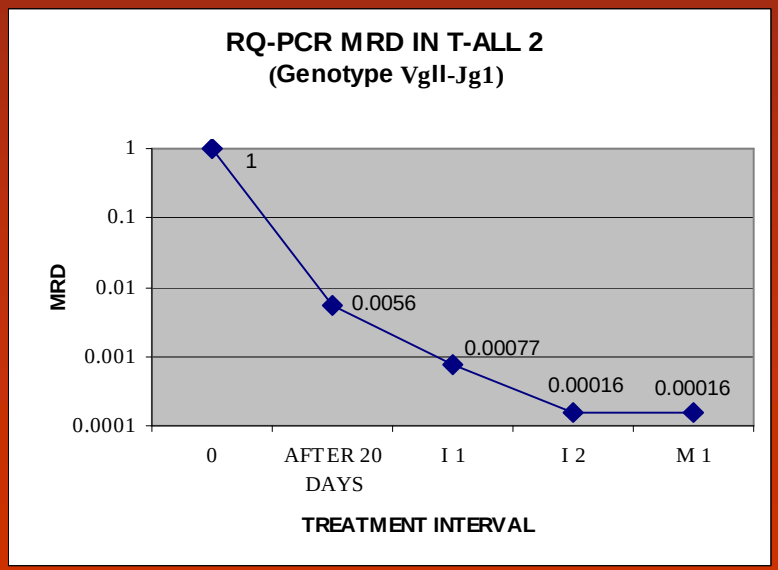
Slope –3.631192
Intercept 38.376797
R2 = 0.962376

MRD Quantitation in ALL patients









Conclusion

- *TCRG* rearrangements were detected in 68.5% and *TCRD* in 29.6% of the patients
- After Induction therapy, in 3 of the 4 patients lesser than 2 leukemic cells in 10^3 normal cells were present and those patients are in clinical and hematological remission
- MRD quantitation with more number of samples before and after treatment are required to risk stratify the patients
- Acknowledgements

  - Department of Science and Technology, New Delhi.
  - Lady Tata Memorial Trust, Mumbai and
  - Department of Medical Oncology, Cancer Institute.