Loss of Heterozygosity detected in a short tandem repeat and confirmed by NGS-based chimerism: a case report

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BACKGROUND

- Hematopoietic stem cell transplantation (HSCT) is increasingly used for hematologic malignancies, bone marrow failure syndromes, and inherited disorders such as sickle cell disease, thalassemia, and immunodeficiencies.
- Advances have improved safety, but risks remain, including: relapse, infection, graft-versus-host disease (GVHD), immunodeficiency, and graft failure.
- Chimerism analysis is central to post-HSCT monitoring. It assesses donor versus host hematopoiesis and helps determine engraftment success.
- Chimerism testing can reveal early relapse through detection of residual host cells.
- Subset-specific chimerism analysis provides greater sensitivity and clinical insight.

METHODOLOGY

Analysis of short tandem repeats (STRs) has long been the predominant method of chimerism analysis. In addition, novel techniques such as next-generation sequencing (NGS) systems have emerged. The result is reported as donor chimerism (donor %).

RESULTS

We report here a case of an 11-year-old female diagnosed with ALL who underwent an HLA-identical sibling HSC Transplant. Initially the patient achieved chimerism with successful engraftment at post-operative days 23 and 50. However, by day 63 post-HSCT, the genotype showed a mixture of donor and recipient, signaling an aggressive relapse trajectory **(Fig. 1A)**. By day 91 a mixed chimerism characterized by a resurgence of recipient genotype in the Neutrophil, NK, and B-cell lineages declined to 70%, 50%, and 2% donor chimerism respectively**,** consistent with early relapse. Interestingly, by day 63 post-HSCT chimerism analysis using STR markers revealed early Loss of Heterozygosity (LOH) in the B-cell lineage. By day 91, LOH had expanded to the neutrophil and NK cell lineages. The pretransplant recipient STR genotype showed two informative alleles at TPOX and D2S1338 loci; the second recipient allele at TPOX (9) and D2S1338 (20) are missing in the B-cells, Neutrophils and NK cells lineages **(Fig. 1B**). To confirm the LOH, the patient HLA genotype was retested by NGS using DNA isolated from purified B-cells. In parallel, NGS-based chimerism analysis was performed using 202 single nucleotide polymorphisms (SNPs) profiling. The results demonstrated a loss of an entire haplotype, with LOH affecting multiple SNPs loci across the genome **(Fig. 2A, B)**.

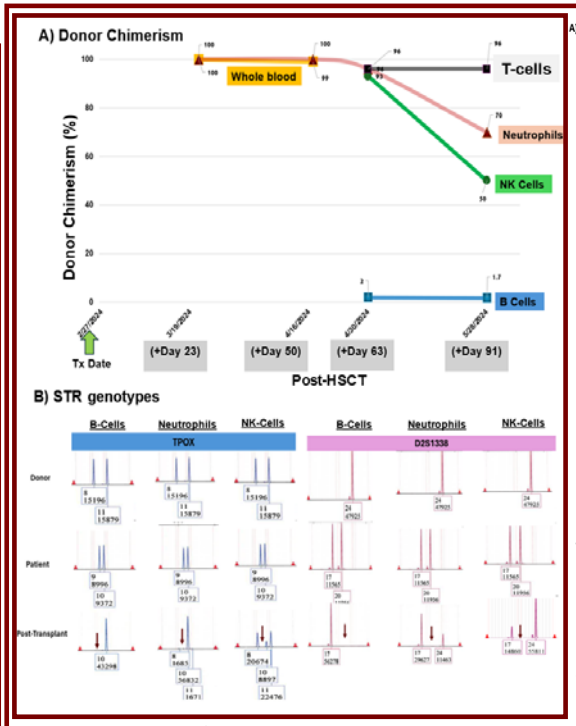

Figure 1A, B: A) Donor chimerism percentages across whole blood, Neutrophil, T, NK, and B-cell lineages at multiple time points post-HSCT. B) Chimerism analysis using STR markers. Arrows indicate loss of the recipient informative alleles; TPOX (9) and D2S1338 (20)

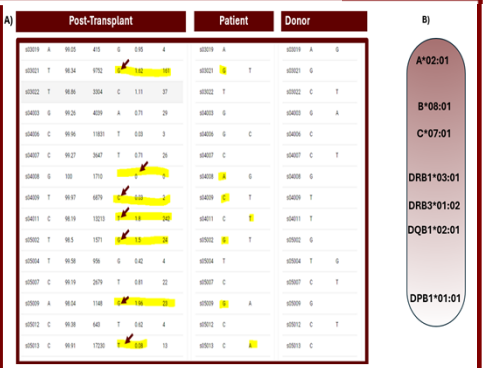

Figure 2A, B: LOH confirmation by NGS-based chimerism analysis and NGS-based HLA genotyping using DNA isolated from purified B-cells. A) NGS confirmed LOH with SNP profiling affecting multiple loci across the genome. Arrows indicate examples of 7 SNPs showing loss of the recipient informative and semi-informative alleles. B) HLA retyping demonstrated a loss of an entire HLA haplotype in B-cell lineage.

CONCLUSIONS

1. The case first illustrates that the detection of early LOH should prompt consideration of preemptive interventions, such as donor lymphocyte infusion or targeted immunotherapies, to counteract immune escape and clonal expansion.
2. In addition, this finding is significant because LOH of HLA alleles may enable leukemic cells to escape immune recognition by donor-derived T and NK cells, effectively circumventing the GVL effect.