Chimerism Monitoring of Post-Allogeneic Hematopoietic Stem Cell Transplantation by Next Generation Sequencing

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Hematopoietic stem cell transplantation (HSCT) is the gold standard treatment for most haematological diseases. Post allogeneic-HSCT chimerism analysis is used to evaluate donor cell engraftment and early detection of relapse. We aim to evaluate the performance of a Next-Generation Sequencing chimerism test and compare it to the quantitative real-time PCR (RT-PCR) method. We use AlloSeq HCT, a multiplexed one-step PCR-based solution. This assay utilizes the difference in single nucleotide polymorphism (SNP) loci to measure the percent DNA fraction relative to total amount of DNA from post-transplant sample. Genomic DNA was derived from five donor-recipient paired samples at day 30, day 60 and day 90 post-transplant. Libraries are sequenced on the MiSeq System (Illumina, Inc). Once completed, the percentage DNA fraction of up to 3 genomes present in each sample is calculated using the AlloSeq HCT software. For test validation, we run the same samples in parallel with RT-PCR method using GenDX®KMRtrack kit. The results showed a high concordance between both methods (correlation of $\geq$ 0.99). Lower limit of quantification (sensitivity of the NGS test) was 0.3% for a single donor. The AlloSeq HCT assay detected percent recipient of donor DNA in the range between 0.1- 99.88% in the panels with up to two or three genetic contributors. In conclusion, NGS platform for chimerism monitoring shows high performance and sensitivity. Further validation studies are needed to integrate NGS in clinical routines of chimerism monitoring.

Keywords: Hematopoietic stem cell transplantation (HSCT), Next-Generation Sequencing

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