FLT3-ITD Monitoring Using Next-generation Sequencing in Acute Myeloid Leukemia

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Background: Internal tandem duplication in the FLT3 gene (FLT3-ITD) is an important negative prognostic marker in acute myeloid leukemia (AML). In addition, FLT3-ITD monitoring is particularly important in patients at relapse or receiving FLT3 targeted therapies. However, current FLT3-ITD testing methods, based on PCR-fragment analysis (FA) or sanger sequencing are insensitive for minimal residual disease (MRD) monitoring. Therefore, we developed the NGS-based FLT3-ITD monitoring platform to improve detection sensitivity and validated its prognostic power using clinical samples.

Materials and Method: We enrolled 39 AML patients with mutated FLT3-ITD and received allo-HSCT at Seoul St. Mary’s Hospital. Total of 168 bone marrow samples collected at the time of diagnosis and during the course of treatment were used. For the NGS based assay, three sets of primers covering exon 13-15 were used for paired-end sequencing by NextSeq system. Clinical information about the use of FLT3 inhibitor, and ELN risk status were obtained through chart review. We analyzed the predictive powers of the five variables for relapse and survival: initial burden of FLT3-ITD mutation, MRD status before and after allo-HSCT, use of FLT3 inhibitor and ELN risk status.

Results: Among the 168 samples, FA and NGS assay identified ITDs of various lengths (18bp-81bp) in 44 samples at the time of diagnosis or relapse. Among the 124 samples with negative MRD by FA, NGS based assay additionally detected ITDs in 39 samples at before and/or after HSCT (Median VAF: 0.000468, 95% CI: 0.000133-0.001403). Survival analysis revealed that the positive MRD after HSCT was the most powerful predictor for relapse (P=0.0017, Hazard ratio: 12.68, 95% CI: 2.60-61.93) followed by use of FLT3 inhibitor (P=0.0113) and pre-HSCT MRD (P=0.0373). The use of FLT3 inhibitor appeared to influence the prolonged survival (P=0.0034, Hazard ratio: 0.20, 95% CI: 0.07-0.59). Conversely, the ELN risk stratification and initial allelic ratio (>0.5) were not significantly associated with the outcome. The multivariable analysis demonstrated that the MRD status after HSCT and the use of FLT3 inhibitor were significantly related with the relapse (P=0.004, Hazard ratio: 25.19, 95% CI: 2.79-227.35) and survival (P=0.0347, Hazard ratio: 0.10, 95% CI: 0.01-0.85), respectively.

Conclusion: We demonstrated the NGS based method is more sensitive in detection of FLT3-ITD mutational burden than the conventional methods such as FA. The MRD status analyzed by NGS could predict the relapse more precisely than the conventional ELN classification. We also found that the FLT3 inhibitor had favorable effect on the survival. Collectively, highly sensitive MRD monitoring of FLT3 ITD must be incorporated into AML management to guide clinical decisions. The implementation of this method in routine diagnostics may contribute to revising future treatment guidelines and goals. Our method is easy to apply in the clinical laboratory and could be a possible alternative to FA for FLT3-ITD MRD monitoring in the era of personalized medicine.

Keywords: MRD, AML, FLT3-ITD, NGS, Fragment analysis