Identification of Somatic Mutations in Acute Myeloid Leukemia Patients Using the TruSight Myeloid Sequencing Panel

Juli-Anne Gardner, MD, CG(ASCP)CM, Francine de Abreu, PhD, Jason Peterson, MS, Gregory Tsongalis, PhD, Prabhjot Kaur, MD, Dartmouth-Hitchcock Medical Center

Somatic mutations in myeloid malignancies correlate with WHO classification, help define genotype/phenotype relationships of clinical relevance, and are an essential part of the diagnostic algorithm. Current methods of detection are timely, costly, and labor intensive, and only assess single genes. The TruSight Myeloid Sequencing Panel (TSMSP) targets 54 genes frequently mutated in myeloid malignancies. This study evaluates the TSMSP. Eight samples, previously tested for genetic variants, were sequenced using the TSMSP: 6 AML patients and 2 controls (Horizon, Tru-Q NGS DNA Reference Standard 4 - 5% Tier; Illumina, ACD1). Assays were performed according to manufacturer’s recommendations. For library preparation, oligo primers were hybridized to targeted regions present in 50 ng of gDNA, followed by extension and ligation. Indices and sequence adapters were added by PCR amplification. Finally, libraries were purified, normalized, pooled, and sequenced on the Illumina MiSeq system. Base-calling and sequence alignment were performed using MiSeq reporter software. VCF files were generated using Somatic Variant Caller and uploaded to VariantStudio v2.1, where variants were annotated, classified, and filtered for quality, coverage, read depth, allelic frequency, and significance. The TSMSP showed 100% concordance in variants identified within genomic regions previously tested. Variant calls between assays had similar allelic frequencies. One sample previously identified as wild type presented with a mutation in ASXL1, which is associated with shorter overall survival. Two samples shared the same NPM1 insertion (p.W288fs), and two had NRAS point mutations (G12R, G13R). Samples with NPM1 insertion presented with additional variants, including one with an FLT3 insertion and another with an FLT3 and IDH2 point mutation (R140Q). Two samples presented with the same IDH2 mutation. Detection of mutations associated with myeloid malignancies may lead to early diagnosis, as well as appropriate treatment and better clinical outcomes. TSMSP offers a robust NGS-based assay for assessment of AML cases.

© American Society for Clinical Pathology

Am J Clin Pathol 2015;144:A238