Validation of a 54 Gene Myeloid NGS Panel Using an Independent Tertiary Analysis Software Program

Introduction: Sequencing DNA of tumor cells from patients with myeloid neoplasms has therapeutic, diagnostic, and prognostic implications. Presented here are validation data combining one commercial sequencing method with an independent commercial tertiary analysis software program. **Methods**: DNA extracted using either a manual or automated method from 13 peripheral blood and 38 bone marrow specimens from patients with a myeloid malignancy was sequenced. Additionally, 15 CAP PT specimens and 5 commercial standards (Horizon Tru-Q 1-4, Horizon OncoSpan) were analyzed. Sequencing was performed on 2 Illumina MiSeq Dx instruments using the amplicon-based Illumina TruSight Myeloid 54-gene Sequencing Panel. Genome.vcf files were generated in the Illumina DNA Amplicon app with alignment to the hg19 reference genome. These files were then uploaded into the Qiagen Clinical Insight Interpret software for tertiary analysis using custom filters. All results were compared to those obtained from reference labs (patient specimens) or CAP PT results/known controls. **Results:** The 71 samples resulted in 186 detected variants consisting of 147 SNVs, 37 indels, and 2 MNVs. For the indels, detected size range was 1-66 bp for insertions and 1-52 bp for deletions. Detection of the 52 bp type-1 *CALR* deletion required secondary analysis with the Illumina BaseSpace Pindel app. Analysis of *FLT3* and *CEBPA* variants were not evaluated in the present study as they may not be amenable to the present NGS methodology secondary to large duplications or being GC-rich, respectively. In total, 185 positive variant calls and 1173 negative variant calls matched expected results, with 1 false negative and 2 false positives, yielding a sensitivity of 99.5%, a specificity of 99.8%, a PPV of 98.9% and an NPV of 99.9%. The one false negative was due to the variant being in a region with strand bias and thus was not called. Orthogonal analysis was not performed on the 2 potential false positive variants and thus their true presence or absence could not be confirmed. The mean difference in VAF between the present study and the reference labs was 2.5% $\pm$ 3.3% (S.D.) with 98% of variants within 10% VAF. Lastly, the overall clinical interpretation (e.g., Tier classification, predicted response to therapy, etc.) of all variants were concordant with those reported by the reference labs in patient specimens. **Conclusions:** The combination of the Illumina Trusight Myeloid Sequencing Panel and the Qiagen Clinical Insight Interpreter tertiary analysis software is a sensitive and specific approach for the detection and interpretation of clinically important variants in myeloid neoplasms.