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617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Validation of an Automated, Scalable Comprehensive Genomic Profiling Assay for Hematologic Malignancies

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Introduction:
The optimal diagnosis, prognosis, and treatment selection for hematologic disorders requires the assessment of somatic mutations across a subset of clinically relevant genes. Here we present the clinical validation results of a targeted next generation sequencing panel comprising all exons of 141 clinically relevant genes for myeloid and lymphoid malignancies.
Methods:
The panel was designed to include genes (n=141) based of clinical guidelines for myeloid diseases including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN), and lymphoid diseases including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and some lymphoma subtypes. The panel was validated to detect single nucleotide variants (SNVs), insertions/deletions (indels), *FLT3* internal tandem duplications (*FLT3*-ITDs), and sex.
Gene level copy number alterations (CNAs) were validated for 11 genes and 5 sub-gene level CNAs in *IKZF1*, *KMT2A*, *RUNX1*, *TET2*, and *TP53*, and were selected based on clinical utility and frequency observed in a population of >10,000 microarray samples with confirmed myeloid or lymphoid indications.
Custom hybridization probes (Twist Biosciences) were designed to interrogate all coding exons of the 141 genes using genomic libraries created from 250 ng of gDNA extracted from peripheral blood, bone marrow, or flow cytometry cell suspensions, followed by hybrid capture, and sequencing on Illumina DNA sequencers. Data analysis was performed using an in-house analysis pipeline to detect variants and perform rational filtering of technical artifacts.
Validation studies were performed in a CAP/CLIA accredited clinical laboratory. Precision and concordance studies were performed using previously characterized clinical samples. SNVs, indels and *FLT3*-ITDs were orthogonally confirmed by an NGS-based laboratory developed test (ArcherDx) or by Sanger sequencing. CNAs were confirmed by digital multiplexed ligation-dependent probe amplification (dMLPA; MRC Holland).
Results:
Assay precision was determined using 15 previously characterized clinical samples with 36 SNVs with a variant allele frequency (VAF) range of 5.7-91.4%, 11 indels with a VAF range of 6.2-79.7%, one 24bp *FLT3*-ITD with a VAF of 30.2%, 17 deletions with a copy number (CN) range of 0.53-0.83, and 7 gains with CN range of 1.32-1.66. Three replicates of each sample were assessed for precision with 100% concordance observed for SNVs (36/36), indels (11/11), *FLT3*-ITD (1/1), CNAs (24/24), and gender (15/15). Inter-assay precision was assessed using multiple operators, instruments, reagent lots, and sample barcodes with the clinical samples.
Interim analysis of concordance performed on 130 clinical samples demonstrated a positive percent agreement (PPA) of 99.7% for SNVs (334/335 in 114 clinical samples), 97.7% for indels (146/151 in 85 clinical samples), 98.7% for CNAs (30/30 in 17 clinical samples), and 96.7% for sex (127/130 in 130 clinical samples). Dilution series of both cell lines and clinical samples that will be used to determine analytical sensitivity are underway.

Conclusions:
This study highlights the clinical validation of a 141 gene NGS assay for the detection of clinically informative genomic alterations in hematologic malignancies. The detection of CNAs by NGS may be used to additionally confirm or monitor abnormalities previously detected by cytogenetics/microarray or inform the need for additional testing. Results from this validation study, when complete, will include at least 480 samples plus orthogonal testing. These data describe the performance of an assay to enable a comprehensive evaluation of genomic alterations using a single sample, further facilitating the use of broad NGS assays in hematologic malignancies.

Disclosures Hogg: *Labcorp:* Current Employment. **Liu:** *Labcorp:* Current Employment. **Cao:** *Labcorp:* Current Employment. **Shafi:** *Labcorp:* Current Employment. **Shabaneh:** *Labcorp:* Current Employment. **Howitt:** *Labcorp:* Current Employment. **Williamson:** *Labcorp:* Current Employment. **Dango:** *Labcorp:* Current Employment. **Guan:** *Labcorp:* Current Employment. **Hoffmann:** *Labcorp:* Current holder of *stock options* in a privately-held company, Ended employment in the past 24 months. **Mooney:** *Labcorp:* Current Employment, Current holder of *stock options* in a privately-held company. **Pruitt:** *Labcorp:* Current Employment. **Parker:** *Labcorp:* Current Employment. **Dong:** *Labcorp:* Current Employment, Current holder of *stock options* in a privately-held company. **Letovsky:** *Labcorp:* Current Employment, Current holder of *stock options* in a privately-held company. **Cai:** *Labcorp:* Current Employment, Current holder of *stock options* in a privately-held company. **Ramkissoon:** *Labcorp:* Current Employment, Current equity holder in publicly-traded company. **Chenn:** *Qiagen:* Honoraria; *Labcorp:* Current Employment, Current holder of *stock options* in a privately-held company. **Eisenberg:** *Labcorp:* Current Employment, Current equity holder in publicly-traded company, Current holder of *stock options* in a privately-held company. **Almasri:** *Labcorp:* Current Employment, Current holder of *stock options* in a privately-held company. **Jensen:** *PetDx:* Consultancy; *Labcorp:* Current Employment, Current equity holder in publicly-traded company, Current holder of *stock options* in a privately-held company, Patents & Royalties.

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