H44. Minimal Residual Disease Detection of Lymphoid and Plasma Cell Neoplasms Using a Next-Generation Sequencing (NGS)-Based Assay

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Introduction: Lymphoid and plasma cell neoplasms are characterized by clonally-restricted T-cell receptor (TCR) or immunoglobulin (Ig) rearrangements. Across clinical laboratories, this is generally demonstrated with standardized multiplex polymerase chain reaction (PCR) assays, in which V-J or D-J products are separated by fragment sizes on capillary electrophoresis (CE). However, this approach has relatively low sensitivity and does not provide the specific clonal sequence information required for tracking a clone at low level or in minimal residual disease (MRD) setting. In this study, we assessed the performance of a NGS-based assay, LymphoTrack (LT) (Invivoscribe), for detection of low level and MRD in comparison to CE and flow cytometry (FC) assays. **Methods:** DNA was extracted from bone marrow, blood, and formalin-fixed paraffin-embedded tissue from 30 patients with diagnostic and post-therapy (PT) samples. For clonal Ig rearrangement, PCR primers flanking the *IGH* conserved framework region 1 (FR1) in VH and conserved JH region were used. For clonal TCR rearrangement, primers flanking the *TRG* conserved Vγ and Jγ regions were used. The amplified products were sequenced on the Illumina MiSeq platform, and analyzed with the proprietary LymphoTrack analysis software, which provided the quantitation and V-J gene family usages of all unique sequences. The patient-specific diagnostic clonal sequences were used to detect MRD in subsequent samples, and compared to concurrent CE and FC results. **Results:** The study included 30 diagnostic and 37 PT samples, from patients with plasma cell neoplasms (PCN) (n=9), acute lymphoblastic leukemias (n=7), mature B-cell neoplasms (n=8), and mature T-cell neoplasms (n=6). Median number of sequencing reads for PT samples was 440,600, with the expected clonal sequences detected in as low as 0.0045% of reads. In 20/37 (54.1%) PT samples, MRD was detected by CE and/or FC; LT detected MRD in 19/20 of these cases (sensitivity=95%). LT did not detect MRD in a case of PCN, but FC detected 0.0028% plasma cells suspicious for disease. In 8/37 (21.6%) PT samples, only LT detected MRD, whereas FC and CE failed to detect any disease. In 11/37 (29.7%) PT samples, both LT and FC detected MRD, whereas CE did not. In 2/8 cases (25%) with MRD detected by LT only, subsequent patient samples showed overt disease with a median follow-up of 2.5 months. 7/37 (18.9%) PT samples were negative for MRD by all assays, and 6/7 (85.7%) of these patients remained disease-free with a median follow-up of 3.5 months. **Conclusions:** Compared to CE and FC, LymphoTrack provides comparable or better MRD detection sensitivity of lymphoid neoplasms, and with increased diagnostic certainty by utilizing patient-specific clonal sequences for MRD detection.

ID44. A Laboratory-Developed Metagenomic NGS Assay for Pathogen Detection in CSF Specimens

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Introduction: Metagenomic next-generation sequencing (mNGS) for pan-pathogen detection has been successfully tested in proof of concept studies to aid in difficult diagnostic cases, but is not available in a clinical setting to date. The UCSF Clinical Microbiology Lab has developed and validated a clinical mNGS assay for diagnosis of infectious causes of meningitis and encephalitis from cerebrospinal fluid (CSF). We evaluated performance characteristics of the assay using contrived and excess patient samples. **Methods:** Two libraries per sample were constructed from DNA and RNA portions of extract and prepared using Nextera XT, with 5-20 million sequences generated per library. Internal controls consisting of DNA and RNA phage particles were spiked in prior to extraction. An external positive control made of 7 representative organisms (DNA virus, RNA virus, Gram-negative rod, Gram-positive coccus, yeast, mold, and parasite) was developed. Positive control dilutions in synthetic CSF were used to determine limits of detection. To document the effects of inhibition, hemolyzed RBC and human DNA and RNA were added prior to extraction to the positive control and sequenced. The accuracy study included 105 CSF samples (known positives: 28 DNA viral, 11 RNA viral, 5 bacterial, 14 fungal, and 1 parasitic). For bioinformatics analysis, the SURPI pipeline for pan-pathogen detection from mNGS data was optimized to report positives within established cutoffs after normalization (SURPIclin). An algorithm for automatic reporting of detected pathogens was developed with final interpretation by laboratory physicians. Discrepancy testing was conducted when possible using additional orthogonal molecular tests when mNGS detected organisms not routinely tested for by reference methods, and to establish the presence or absence of microorganism nucleic acid. **Results:** We established quality metrics, threshold values, and limits of detection of between 0.01-130 genomic copies or colony forming units per mL. Gross hemolysis and high white blood cell counts reduced assay sensitivity; however, the phage-spiked internal control was a reliable indicator of sensitivity loss. The assay exhibited 76.4% sensitivity and 98.3% specificity compared to reference lab results. 27/87 (31%) of organisms undetected by mNGS were rare isolates by culture or Ct >37 by qPCR; when removed from the accuracy study, assay sensitivity improved to 90.9%. **Conclusions:** A mNGS assay for pan-pathogen detection in the context of samples from patients with encephalitis or meningitis was developed and clinically validated. Performance characteristics are highly promising. Clinical utility is being evaluated in a 300-patient prospective study supported by the California Initiative to Advance Precision Medicine.

I22. Application of Data Visualization for the Clinical Interpretation of Next-Generation Sequencing (NGS)-Based Assay Results

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Introduction: Determining the clinical significance of any given sequence variant requires an integrated evaluation of information from multiple genomic data sources, such as variant location mapping, predicted cDNA and amino acid sequence change, minor allele (population) frequency, insilico prediction scores, somatic and germline mutation databases, and comparison with previously identified variants (in-house knowledgebase). Software tools to visualize the mapping of sequence variant to peptide structure have been developed and used predominantly in research projects. We investigated the scope for applicability of data visualization techniques to assist in the interpretation of sequence variants from NGS-based somatic mutation testing. **Methods:** We developed a modular, browser-based visualization widget using JavaScript using widely-used, freely-available libraries such as jQuery. The widget can consume publicly-available or custom web services via AJAX (Asynchronous JavaScript and XML) to obtain data for visualization for a variety of annotations. The interactive graphical user interface utilizes HTML, SVG and CSS, and is encapsulated in an HTML element for easy integration with any web application. An application programming interface (API) provides developers with a variety of configuration options for programmatic control over the widget. Intuitive user interface and easy integration into existing clinical workflows were important design considerations. **Results:** The data visualization widget was successfully incorporated into a clinical variant management and reporting application used at our institution with minimal change in codebase. It rendered an integrated visualization of the variant location, functional domain mapping, and details and prevalence of a variant of interest when compared to in-house knowledgebase or external databases. For example, during review of uncommon or rare somatic variants, the widget provided visual overlay of the existing variant with variants from the COSMIC database while preserving the positional mapping to the functional protein domains. During routine clinical case workup, it minimized the requirement for separate review of multiple data sources such as pFam, uniprot, COSMIC and other data sources. **Conclusions:** Data visualization is powerful approach for presenting and interpreting complex genomic data, in clinical as well as research settings. We developed a modular, browser-based widget, which was easily configurable and incorporated into our existing clinical application. Integrated data visualization provided a novel and valuable user experience for variant interpretation, especially with high test volumes.