H046. The Clinical Validation of a Targeted Next-Generation Sequencing Panel for Lymphoid Malignancies

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Introduction: Lymphoid malignancies are a heterogeneous group of hematological disorders traditionally characterized by a diverse range of morphological, immunophenotypic, and clinical features. In recent years, large-scale sequencing efforts have identified important genetic markers in lymphoma that augment these standard methods of characterization. Here we report the clinical validation of a custom capture-based next-generation sequencing (NGS) panel to test for molecular markers in a range of mature lymphoid and histiocytic neoplasms. Methods: A custom panel was designed using xGen Lockdown Probes (Integrated DNA Technologies) to tile across 77 genes selected for their clinical relevance in lymphoid and histiocytic malignancies. A cohort of 199 unique samples (fresh/frozen and formalin-fixed, paraffin-embedded [FFPE]) were used to assess accuracy, limit of detection (LOD), reproducibility, and specificity. DNA was isolated using a Promega extraction and prepared for NGS using a KAPA HyperPrep protocol (Roche) and a capture-based enrichment. Sequencing was performed on the NovaSeq 6000 (Illumina), and data were analyzed using an internal bioinformatics pipeline. Variant calling was performed using TNhaplotyper2 (Sentieon) for single nucleotide variants (SNVs) and small indels and Manta (Chen, et al., Bioinformatics, 2016) and Pindel (Ye, et al., Bioinformatics, 2009) for larger indels up to 1000 bps. Ion Proton sequencing (Thermo Fisher Scientific) was utilized as an orthogonal method of confirmation. Results: More than 50% of the samples included on the validation were FFPE derived (94 non-FFPE and 105 FFPE) to ensure high performance from the lowest quality source of DNA input. Overall, the concordance of reportable SNVs and indels with orthogonal methods was 99.8% (586/587) and 100% (128/128), respectively, at a variant allele frequency (VAF) ≥5.0%. Sixty-nine genes in the 77-gene panel contained at least one reportable variant. The most frequently mutated genes included KMT2D (7%), TP53 (6%), BCL2 (5%), and TET2 (5%). The LOD was determined to be at least 3.0% VAF using Genome in a Bottle (GIAB) cell line mixes and Horizon Discovery FFPE material. However, inter-run and intra-run reproducibility were highest for variants ≥5.0% (both 99.9%) compared to variants ≥3.0% (95.0% and 96.1%, respectively). Specificity was determined to be 100% using two GIAB standards. Conclusions: This validation establishes the robust performance of a novel targeted NGS panel for the accurate and sensitive detection of SNV and indel variants in both FFPE and non-FFPE sample types. The panel demonstrates the capability to identify clinically relevant and actionable mutations to facilitate the characterization and management of patients with lymphoid and histiocytic neoplasms.
Introduction: Diagnosis of central nervous system lymphoma (CNSL) often requires neurosurgical biopsy due to the low sensitivity of cerebrospinal fluid (CSF) cytology. High-sensitivity molecular profiling of MYD88 status in cerebrospinal fluid (CSF), which has been implicated in the differential diagnosis of primary CNSL and Waldenström macroglobulinemia, allows for minimally invasive and fast diagnosis of CNSL. Here we outline an interdepartmental effort to translate a rapid genotyping assay for the detection of MYD88 in CSF into a laboratory-developed test for use in a CLIA-certified laboratory.

Methods: A highly sensitive MYD88 genotyping assay was developed to detect the MYD88 c.794T>C/L265P missense mutation and optimized using wild-type clamping peptide nucleic acid oligonucleotides for analysis of nucleic acids (NA) derived from CSF. Assay performance was assessed in n = 18 CSF specimens and n = 21 supplemental formalin-fixed, paraffin-embedded (FFPE) and bone marrow (BM) specimens. Because the fraction of circulating tumor DNA in CSF is extremely low and likely exists in the extracellular matrix, we separate the CSF into supernatant and cell pellet. NA from the CSF is then isolated using different extraction kits (circulating cell-free DNA and FFPE) and independently tested for MYD88 L265P. Specificity was assessed in n = 12 CSF samples with known MYD88 wild-type status, and sensitivity was assessed in n = 5 CSF specimens with known MYD88 L265P status. Limit of detection (LOD) was determined in n = 1 FFPE and n = 1 BM with known mutant allele fraction serially diluted in genomic DNA. LOD experiments were performed twice to assess inter-run reproducibility. FFPE specimens were run in duplicate to assess intra-run reproducibility.

Results: Diagnostic specificity, sensitivity, and positive and negative predictive values were 100%. LOD by allele fraction was 0.23% in FFPE and 0.24% in BM. All technical replicates and repeats were reproducible (100% precision). Since the clinical launch, 48 CSF specimens have been tested with an average turnaround time of three days and a range of zero to six days from the date of accessioning to report generation. Among four clinical cases positive for MYD88 L265P (8.3%), the most notable diagnosis was confirmed only in the cell-free component of a specimen where traditional testing failed to detect the variant.

Conclusions: Performance metrics of this rapid MYD88 assay were indicative of analytical validity, and the assay was incorporated into existing clinical workflows. The clinical utility of a rapid cell-free DNA-based assay is the detection of MYD88 L265P in CSF to refine diagnosis to lymphoproliferative malignancies, potentially obviating the need for invasive intracranial biopsies. We implemented standard operating procedures, competency, and proficiency assessments in accordance with CLIA.
TT066. Melanin Bleaching for Biocartis Idylla BRAF Mutation Assay for Pigmented Melanoma

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Introduction: Next-generation sequencing (NGS) has identified multiple molecular alterations in metastatic melanoma that are candidates for targeted therapy, such as BRAF gene mutations present in 40% to 60% of cases. Identifying BRAF mutations in rare, heavily pigmented melanoma is essential; however, excessive melanin pigmentation negatively affects histologic examination and inhibits PCR reaction. Effective melanin-bleaching method is necessary to improve the diagnostic accuracy and quality of DNA extraction of heavily pigmented melanomas. Whereas NGS methods are difficult to modify due to the multiple steps involved, cartridge-based BRAF tests allow for melanin bleaching by preserving the specimen quality, as specimen preparation is the only variable in the process. Methods: BRAF mutation positive and negative formalin-fixed, paraffin-embedded melanoma tissues were retrieved. Tissues with varying degrees of melanin pigmentation were selected after hematoxylin and eosin (H&E) slide review. Sections were cut at 4μm, air dried, and then treated with a 5% or 10% dilution of H₂O₂ for 10, 20, or 30 min at 65°C and rinsed for two min with nuclease-free water. Both untreated and treated sections were H&E stained to confirm adequate tissue preservation and depigmentation. To examine nucleic acid preservation, treated and untreated sections were stained for S100 and Sox-10 by immunohistochemistry (IHC). Both conditions were tested for BRAF mutation using the Biocartis Idylla system.

Results: Best bleaching was achieved with 10% H₂O₂ at alkaline pH and 65°C for 30 min. With this bleaching, decreased Cq number, indicative of a more reliable result, was obtained while maintaining nuclear integrity and improving the morphology. Post-treatment test results for Biocartis Idylla BRAF mutation had a 100% correlation with NGS results. Due to the rarity of heavily pigmented melanomas, no NGS-negative post-bleach Idylla-positive case was identified. Conclusions: Melanin bleaching can be a double-edged sword: The melanin may disappear, but the tissue's morphology and integrity of nuclear contents may be compromised. KMnO₄ frequently compromises structural integrity and antigenicity in IHC. Thus, a gentler H₂O₂ treatment was selected for the molecular analysis of moderately/heavily pigmented melanomas. Post-bleaching assays showed lower Cq results than non-treated tests, indicating decreased melanin inhibition. The 10% H₂O₂ solution was highly effective in bleaching melanin without compromising morphologic, immunologic, and molecular tests. Maintaining basic pH is critically important for optimal assay performance. This modified melanin bleaching for Idylla BRAF assay allows prompt management decisions for heavily pigmented advanced melanomas with same-day turnaround time.