Validation and Implementation of Long Mononucleotide Repeat Testing for the Detection of Microsatellite Instability: A Single Institution Experience

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Introduction: Microsatellites are short, repetitive portions of DNA that are error prone during replication; several mismatch repair proteins recognize and repair these errors. Deficient MMR (dMMR) results in microsatellite instability (MSI), a well-recognized carcinogenic pathway. In Lynch syndrome (LS), germline mutations in MMR genes predispose to colorectal (CRC) and endometrial carcinoma (EC), among others. Immune checkpoint inhibitors (ICI), such as pembrolizumab, are approved to treat dMMR or MSI-high (MSI-H) cancers regardless of tumor type. Therefore, sensitive and specific methods to detect MSI are of critical importance to patient care. Multiple methods are used to detect MSI status, including immunohistochemistry (IHC) for MMR proteins and molecular assays. The Detroit Medical Center utilizes both IHC and PCR-based assays to maximize MSI detection sensitivity. We attempted validation of a new fluorescent PCR-based assay for MSI tumor testing which compares allelic profiles of microsatellite markers from matching normal and tumor samples. The assay utilizes four traditional MSI markers in addition to four long mononucleotide repeat (LMR) markers. LMR markers produce more pronounced fragment length changes, and thus may improve detection of MSI.

Methods: Our validation set included 46 carcinoma patient samples with prior PCR-based MSI and MMR IHC testing. These included MSI-high, MSI-low (MSI-L), and microsatellite stable (MSS) cases. DNA was isolated from formalin-fixed, paraffin embedded (FFPE) tissue and amplified. Stutter peak patterns were analyzed, comparing allele sizes and stutter patterns. All cases were reviewed by two directors, with any discrepancies discussed and reviewed to achieve consensus. MSS samples showed identical stutter patterns between tumor and normal pairs. MSI-L samples had a single discrepant marker, whereas MSI-H samples had 2 or more discrepant markers. The results of the LMR assay were compared with the original PCR-based MSI and IHC results to determine test concordance.

Results: 42 cases were successfully amplified and included in our results. Fourteen cases were classified as MSI-H by consensus, with a range of 3 to 8 unstable markers out of 8 total markers. IHC results for the MSI-H cases were available in 13/14 cases; thirteen were classified as abnormal. Notably, in one EC MSI-H case, a normal IHC result was reported. Three cases were classified as MSI-L. All were CRC samples, and 2 of 3 were also designated MSI-L using the traditional PCR MSI assay. The remaining MSI-L case was formerly interpreted as MSS. The IHC results for the MSI-L cases were all available and reported as normal. The remaining cases were designated as MSS. IHC results were normal in all but one EC case.

Conclusions: Our study validated the LMR MSI Analysis System for detection of MSI; it is now used in clinical practice in our molecular laboratory at DMC, and challenging real-world cases from our assay rollout will be presented graphically. Our validation results demonstrate high concordance (42/42 cases) between the LMR and traditional MSI panels, highlighting the reliability of LMR markers. IHC and PCR results were largely concordant, with occasional discrepancies revealing the importance of a combination approach for MSI detection.