Targeted Genomic Profiling in Colon Cancer: Experience in a Large Community-Based Pathology Practice

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Introduction

NCCN guidelines recommend screening for Lynch Syndrome and assessing for mutations in KRAS, NRAS, and BRAF genes to guide clinical management decisions in the setting of colon cancer. Additional biomarkers have been implicated that could potentially refine diagnosis, prognosis, and/or therapy selection.

Based on NCCN guidelines and literature review, we designed a focused next-generation sequencing (NGS) assay, SmartGenomics™ Colon Panel, to complement our Lynch Syndrome Screening strategy. This panel is designed for use at diagnosis in suspected or proven metastatic colon adenocarcinoma to uncover therapeutic options and treatment planning to improve patient outcomes. Our initial experience with 183 cases utilizing this testing strategy in community practice is presented herein.

Method

The SmartGenomics™ Colon Panel includes 7 genes: KRAS, NRAS, BRAF, PIK3CA, TP53, PTEN and CTNNB1. A total of 42 amplicons covering 4.2 Mb of genomic DNA is sequenced in this panel to detect hotspot mutations.

For formalin fixed solid tissue specimens, areas of the specimen containing tumor were micro-dissected from the surrounding tissue. The microdissected tissue, peripheral blood or bone marrow was subjected to standard genomic DNA isolation procedure (Qiagen QiaSymphony). The genomic DNA was subjected to multiplex PCR amplification of the PathGroup-designed panel (Fluidigm Access Array). The specimen was DNA barcoded for identification purposes and subjected to Next Generation Sequencing (Illumina NextSeq). Bioinformatic analysis (trimming, alignment, variant calling, and variant annotation) was conducted using the MiSeq Reporter Custom Amplicon workflow (version 2.5.1) and the Genome Analysis Toolkit (GATK, version 2.3-9). The presence of questionable variants was confirmed using standard Sanger sequencing (Life Technologies Big Dye) via capillary electrophoresis (Life Technologies 3500x).

The analytic sensitivity of this assay is contingent on the specific mutation, the sequence context of the mutation, and the coverage of the amplicon. In general, the analytic sensitivity should be 5% or greater. The average coverage for the panel is greater than 1,000X. Coverage is amplicon dependent and ranges from 200X to 10,000X within a specimen.

MSI assay by PCR and DNA MMR assay by IHC are also performed in conjunction with the NGS assay as part of colon cancer screening strategy.

Mutation Spectrum

• 183 colon cancer cases were profiled by SmartGenomics™ Colon NGS assay.
• 168 (92%) cases had at least one mutation detected.
• KRAS or NRAS mutation was detected in 81 (44%) patients who would not benefit from anti-EGFR therapies.

Insertions and Deletions

• BRAF mutation was detected in 27 (15%) patients, which is informative to exclude Lynch Syndrome in microsatellite unstable tumors, and identify patients with a grim prognosis for which promising targeted therapies are in active clinical development.
• CTNNB1 mutations were detected in 4 cases (2%) and associated with clinical, morphologic, and pathologic findings highly suggestive of Lynch Syndrome.

Cases with Multiple Mutations

Among 183 cases, 54% had more than 1 mutation, 38% showed 1 mutation and 8% had no mutation detected.

KRAS, NRAS and BRAF Mutations

108 of 168 (64%) mutated cases had KRAS, NRAS, or BRAF mutations detected. Other mutated cases had altered genes but they are not included in NCCN guidelines.

Mutations in KRAS, NRAS and BRAF Wild-Type Cases

60 of 168 (36%) mutated cases that were wild-type for KRAS, NRAS, or BRAF showed mutations in additional genes including PIK3CA, CTNNB1, TP53 and PTEN.

Mutations and MSI Status

• Among 149 cases that had both MSI results and mutations detected, most commonly co-mutated genes are KRAS and TP53. Other pairs include KRAS-PIK3CA, BRAF-PIK3CA, TP53-PIK3CA and BRAF-TP53.
• No cases had both KRAS and BRAF mutations.

MMR Results and Mutations in MSI-H cases

• 18 MSI-H cases also had MMR results. 4 cases showed intact DNA mismatch repair enzyme
  • 3 cases had KRAS mutation detected; 2 of them also had PTEN or TP53 mutations detected. 1 case had no mutation detected.
  • 14 cases had defective DNA mismatch repair enzyme functions
    • 9 cases had loss of DNA mismatch repair enzyme MSH-1 with secondary loss of PM2-2
    • 1 case showed at least 2 mutations; 7 cases had BRAF V600E mutations, thereby excluding Lynch Syndrome; 2 other cases had CTNNB1 S458F/544A mutations, suggestive of Lynch Syndrome.
    • 3 cases had loss of DNA mismatch repair enzyme PM2-1
      • 1 case showed only CTNNB1 S458F mutation; 1 case had no mutations detected; the other case had PIK3CA and TP53 mutations.
      • 2 cases had loss of DNA mismatch repair enzyme MSH-6.

Conclusions

• A focused next generation sequencing panel composed of highly actionable genes provides additional and complimentary information to existing screening assays.
• The 7-gene SmartGenomics™ Colon Panel is well-accepted by community pathologists/oncologists and is feasible for implementation in community practice.
• NGS-based panel allows for simultaneous testing of multiple genomic alterations including insertions and deletions that could be missed by traditional methods.
• CTNNB1 alterations need to be evaluated in larger studies as a potential complement to existing colon cancer testing strategies and a therapeutic target utilizing WNT-pathway inhibitors.
• Further analysis is required to explore the relationship between mutations and MSI status.