413. Stratification of Metastatic Colorectal Cancer Patients Using NGS Sequencing, Neo-Epitope Detection and Immune Infiltrate Analysis

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Introduction
Colorectal cancer (CRC) is the third most common type of cancer in the United States. Although chemotherapy, radiation and targeted therapies can improve survival rates, recent studies have shown the potential benefit of immunotherapies to improve outcomes for patients with advanced CRC. Targeted therapies that use immunomodulatory antibodies (mAbs) to EGFR have been shown to benefit some CRC patients.1 Until recently, KRAS has been the only predictive biomarker for anti-EGFR therapy for metastatic CRC. However, 40% to 60% of patients with wild-type KRAS do not respond to anti-EGFR therapy. Therefore, to accurately predict patients’ response to treatments and improve clinical outcomes, additional prediction and treatment methods are imperative. One of the many efforts to improve prediction for CRC patient’s response to anti-EGFR therapy is the development of gene expression based RAS signature scores for identification of RAS activated tumors independent of mutations in the KRAS gene.2 In addition to passive immunotherapy using mAbs, there have been major advances in targeted active immunotherapy in other tumors, including checkpoint inhibitors and cancer peptide vaccines.3,4 In melanoma, there have been major advances in targeted active immunotherapy in other tumors, including checkpoint inhibitors and cancer peptide vaccines.5

Methods
55 FFPE samples were selected from a cohort of 486 samples with matching FF and flash frozen normal samples. Several driver gene mutations were identified in this study, KRAS, TP53, PIK3CA, APC and HER2. APC splice site mutants account for 126 mutations in the CRC samples.9:

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>No. of Unique Variants in All 53 Samples</th>
<th>No. of Variants Shared by ≥2 Samples</th>
<th>No. of Shared Mutations that Belong to Cancer Genes</th>
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<tbody>
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<td>Non-synonymous SNV</td>
<td>779 mutations</td>
<td>42 mutations</td>
<td>4 mutations</td>
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<td>Splice Site Mutants</td>
<td>128 mutations</td>
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Figure 1. Coefficient of determination (R²) for the number of fusion events detected in samples with lower RAS scores versus samples with higher RAS scores. (A) Distribution of gene fusion events of all samples. Only high and medium confidence gene fusion events based on results from JAFFA were considered. (B) Samples with lower RAS scores have significantly fewer gene fusion events detected than samples with higher RAS scores.

Figure 2. Multi-platform comparison. Samples derived from the same 55 FFPE blocks were assessed across multiple platforms. The method design to combine RNA analysis (gene expression signature scores) with DNA analysis (i.e., mutation status) allows for comparison of RAS signature scores and overall gene expression from different platforms.

Figure 3. Flowchart for the analysis. 55 samples went through 5 different platforms for DNA analysis (i.e., mutation versus KRAS status). The cohort was selected using the following methods: (1) KRAS mutation versus KRAS status. (2) For the second most prevalent driver gene mutations in colorectal cancer both in our data and TCGA data. The second most prevalent cell type in colorectal cancer both in our data and TCGA data. The second most prevalent cell type in colorectal cancer both in our data and TCGA data.

Table 1. Summary of Mutation Identified from Exome seq

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Several driver gene mutations were identified in this study, KRAS, TP53, PIK3CA, APC and HER2. APC mutations could be germline in origin, although this could not be confirmed due to the lack of paired normal samples.

Figure 4. The number of mutation versus KRAS status. KRAS mutant samples have significantly higher number of non-synonymous mutations than KRAS wild type samples.

Figure 5. RAS signature scores and the number of gene fusion events. (A) Distribution of gene fusion events of all samples. Only high and medium confidence gene fusion events based on results from JAFFA were considered. (B) Samples with lower RAS scores have significantly fewer gene fusion events detected than samples with higher RAS scores.

Figure 6. Scatter plot of enriched GO cluster representatives. Multidimensional scaling is applied to the list of significantly enriched GO terms in fusion genes found in the CRC samples.9

Figure 7. Neo-epitope prediction and prioritization.

Figure 8. Work flow for tumor microenvironment analysis. First a content analysis was done using expression of gene signatures associated with each cell type. Since cell type was the most prominent cell type, the immune cell compartment was further stratified into seven different immune cell types using signatures that are specific to each of the following immune cells: CD8 T-cells, CD4 T-cells, T regulatory cells, NK cells, B cells, macrophages and myeloid derived suppressor cells.

Figure 9. Tumor content analysis. RA: RNA-Access (RAseq); RD: ribosomal depletion RAseq; Stromal, Immune and Epithelial scores were generated using gene signatures for each of the three compartments. The expression of the gene signature in the signatures were integrated into a score using ssGSEA (sample Gene Set Enrichment Analysis). Cellular content across different compartments is similar with TCGA data.

Figure 10. Immune phenotyping for CRC samples. RA: RNA-Access (RAseq); ribosomal depletion RAseq. Myeloid-derived suppressor cells are the most prevalent cell type in colon cancer; both for RAseq and TCGA data. The second most prevalent cell type is macrophages. Ribosomal depletion RAseq data is more sensitive than RNAseq. Access data for detecting immune cell types.

References