Abstract
Previously, the mutation status of KRAS was the only validated predictive biomarker for metastatic colorectal cancer (CRC). While KRAS mutated tumors demonstrated resistance to epidermal growth factor (EGFR) inhibitors like cetuximab, KRAS WT and EGFR-expressing tumors were predicted to be responsive. However, KRAS WT metastatic colorectal cancer (CRC) patients have a poor prognosis even with EGFR inhibitor therapy as not all KRAS WT CRCs are responsive to such targeted agents. A gene expression based RAS signature score was developed based on multiple tumor tissue samples to identify RAS activated tumors independent of mutations in the KRAS gene. To further refine this score and define technologies that can be used on FFPE samples isolated in a clinical setting, we analyzed DNA and RNA derived from fifty-five (55) FFPE preserved colorectal cancer tumors biopsies using multiple sequencing, digital and array-based technologies. These samples were selected from a CRC cohort in which the initial gene expression-based RAS signature score was calculated utilizing data compiled from fresh frozen (FF) tumor samples from the same 55 patients. The 55 samples were selected for this study as they had representative samples with high, medium and low RAS signature scores. Transcriptomic analyses (RNA-Seq, Affymetrix® microarrays, Nanoglobe® and Targeted RNA-Seq) were performed on all 55 FFPE samples and three new RAS scores were calculated from the gene expression datasets. These RAS scores were based on different gene signatures; (1) an 18 gene signature, (2) a 13 gene signature, and (3) a 147 gene signature. A significant correlation was identified between RAS scores calculated from the 18 and 13 gene signatures (correlation coefficient –0.88 and –0.76 respectively, r-squared < 0.001). To further refine gene expression signatures, samples were grouped based upon their mutation status obtained by whole exome sequencing (WES) and targeted DNA sequencing data (Illumina TruSeq® and I lureTech Cancer Panels). In our sample set, the 18 gene RAS score was found to be dependent on the mutation status of KRAS. Further analysis is being carried out to better understand the relationship between the calculated RAS signature scores and the mutation status of other genes. This analysis will lead to the development of a novel genomic signature for better pharmacodynamic stratification of colorectal carcinoma patients.

Background
Colonctal cancer (CRC) is the 3rd most common human malignancy and is a major cause of cancer mortality in the Western world. Previously the mutation status of KRAS was the only validated predictive biomarker. Prognosis of metastatic CRC is still poor. Measuring independent biomarkers is unlikely to capture the complexity of RAS signaling pathway dependence. Recent studies have developed RAS signature scores based on a specific gene expression profile.

Methods
Sample Cohort

Cross Platform Comparison

RAS Scores Developed from Gene Expression Signatures
A gene expression signature of RAS pathway dependence predicts response to PI3K and RAS pathway inhibitors and expands the population of RAS pathway activated tumors

RAS Score Calculation
Normalized by Expression of 18 Signature Genes with the Exception of NanoString®

Results

References
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Future Directions

Figure 4. RAS score signature gene expression. Combining gene expression with mutation status of KRAS, NRAS or B-RAF.