Reliable Detection of Low Abundance Somatic Mutations of KRAS, BRAFT, NRAS and PIK3CA in Colorectal Adenocarcinoma Using iPLEX® HS, a New Highly Sensitive Assay for MassARRAY®

INTRODUCTION
The 2016 National Comprehensive Cancer Network Treatment Guidelines (NCCN Guidelines) recommend that patients with metastatic colorectal adenocarcinoma (mCRC) should have tumor tissue genotyped for KRAS, NRAS, and BRAFT mutations. In the United States, many laboratories offer mutation testing using a variety of different platforms with a wide range of analytical sensitivity. Despite considerable progress, analytical challenges remain to be resolved, such as the need for reliable detection of low abundance somatic mutations, particularly in small specimens with a low percentage of tumor cells. In this clinical research study, we assessed 143 patient cases of colorectal adenocarcinoma (CRC) previously tested for mutations in KRAS, NRAS, and BRAFT using a novel analytic approach that reduces wild type signal and allows for detection of low mutation load approaching 1%, the iPLEX® HS for MassARRAY® (Agema Bioscience, San Diego, CA).

METHODS
Archived frozen deoxyribonucleic acid (DNA) samples were searched for human clinical CRC cases previously tested for KRAS, NRAS, and BRAFT mutations using the OncoFocus™ Panel v2.0 or v3.0 and the MassARRAY® system. Specimens were deidentified prior to entry into the study. DNA originated from formalin fixed paraffin embedded (FFPE) tissue samples and all histologic diagnoses were confirmed by a pathologist. DNA was extracted using the QiAamp DNA FFPE Tissue Kit (Qiagen, Boston, MA). Prior to repeat testing, all specimens were assessed for DNA integrity using the iPLEX Pro Sample ID Panel, and all specimens with adequate amplifiable DNA were then interrogated with a new high sensitivity single PCR reaction iPLEX® HS panel that includes more than 34 common mutations in BRAFT, EGFR, KRAS, NRAS, and PIK3CA, both using the MassARRAY® platform. Input DNA requirements for these systems is 10-15ng. For quality assurance an internal positive control was co-detected in all samples. Figure 1 depicts the technical process steps for this system.

RESULTS
The OncoFOCUS™ assay has a mutation detection limit of approximately 5-10% mutant VAF while the iPLEX® HS has increased sensitivity at approximately 1%. In this study we tested 143 CRC patient samples with the iPLEX® HS panel and confirmed all previously identified CRC mutations in KRAS (n=52; 52/143=36.4%), NRAS (n=6; 6/143=4.2%), and BRAFT (n=22; 22/143 = 15.4%). Using the iPLEX® HS chemistry we were able to identify that many mutations in KRAS, NRAS, or BRAFT equating to an increased mutation detection of 5.6%. An example of spectral data comparison from the same sample run on both OncoFOCUS™ and iPLEX® HS is shown in Figure 3. Interestingly, we found that 5 of the 8 new low frequency mutations were in samples with a confirmed higher frequency mutation in another position. However, 3 of the 8 are new mutations for that would have potentially altered the findings/conclusions of the sample analysis. We were also able to identify previously undetected mutations in PIK3CA (n=26; 26/143=18.2%), 17 of the PIK3CA mutations coexisted with other driver mutations, as has been shown by other groups. See Table 1.

CONCLUSIONS
1. In this study our data indicate that lowering assay sensitivity from 5-10% to approximately 1% VAF in clinical CRC cases detected all previously identified mutations in KRAS, NRAS and BRAFT; as well as 8 new mutations in these genes (5.6% increased mutation detection rate).
2. 5/8 of these mutations were in samples with a known higher frequency mutation at another position.
3. 3/8 were a totally new mutation for that sample that would have potentially altered therapeutic decisions for the patient.
4. We also detected 26 PIK3CA mutations (18.2%), of which 17 coexisted with other driver mutations in RAS or BRAFT in 11.9% of CRC cases.
5. In 143 CRC cases, 36.4%, 15.4%, 4.2%, and 18.2% demonstrated a mutation in KRAS, BRAFT, NRAS, and PIK3CA, respectively.

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