NANOFLOWIC DIGITAL PCR FOR IMPROVED SELECTION OF METASTATIC COLORECTAL CANCER PATIENTS TO ANTI-EGFR THERAPIES

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Background: Concomitant detection of mutations in downstream signalling molecules of the EGFR pathway (KRAS, BRAF, PIK3CA and NRAS) has been suggested to improve the selection of candidate metastatic colorectal cancer (mCRC) patients that will respond to anti-EGFR therapy. In addition, EGFR (S492R) point mutation has been associated with acquired resistance to cetuximab. We assessed the feasibility of a nanofluidic digital PCR array platform to simultaneously detect hotspot mutations with high sensitivity.

Methods: 26 primary tumor FFPE tissues from patients (15M/11F; 3 stages I-II; 23 stages III-IV) with chemotherapy-refractory mCRC treated with cetuximab plus chemotherapy in the pre-KRAS selection era (between 1997 and 2006) were included in the mutational analysis. Digital PCR was performed using the Digital Array Chip. Conventional genotyping was performed using LightCycler 480. In both cases TaqMan® probes for wild-type and mutant alleles were used. A panel of 17 hotspot mutations were assessed: 9 in KRAS (G12C, G12V, G12D, G12R, G12S, G12A, G13D, G13C, G13V), 4 in BRAF (V600E, V600K, V600L, V600D), 1 in NRAS (Q61K), and 3 in PIK3CA (E545K, E545G, E545A). The results were compared for concordance and sensitivity of the two methods.

O-0011 Table 1: Clinicopathological characteristics and mutational status of the patients included in the study.
Q61H and A146T), 1 in BRAF (V600E), 4 in exon 20 of PIK3CA (M1043I, H1047R, H1047L and H1047Y), 2 in NRAS (Q61K and Q61R) and 1 in EGFR (S492R).

**Results:** Analytical sensitivity of digital PCR for mutant alleles was 0.05%-0.1% whereas LightCycler detected 1-5%. Eight of 26 (31%) patients were positive for at least one mutation with the LightCycler. Digital PCR increased this number to 11/26 (42%) confirming all positives. Digital PCR identified multiple mutant alleles in 5 cases. Digital PCR reclassified as mutant 1 of the 5 cases with progressive disease. The case with a G13D mutation identified showed partial response (Table 1).

**Conclusion:** Digital PCR provides a robust and highly sensitive detection of EGFR-pathway hotspot mutations that may result in better classification prior to anti-EGFR treatment.