Comparison of enhanced Tagged-Amplicon Sequencing and digital PCR for circulating tumor DNA analysis in advanced breast cancer

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Background
Circulating tumor DNA (ctDNA) analysis allows non-invasive detection of tumor mutations and amplifications in advanced breast cancer. Multiple technologies have been developed to analyse ctDNA. Here we compared two leading ctDNA detection technologies, InVision™ (enhanced tagged-amplicon sequencing) and droplet digital PCR (ddPCR) assays, in advanced breast cancer.

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35 women with advanced breast cancer were recruited to the study. 23 had two separate blood samples taken in a standard EDTA tube processed immediately or in a Streck tube processed up to 120 hours after venipuncture. Droplet digital PCR (ddPCR) was conducted with assays for hotspot actionable mutations in 3 known drivers in breast cancer: PIK3CA exon 9 and 20, ESR1 ligand binding domain (LBD) and AKT1 (c.49G>A; p.E17K), and ctDNA sequencing was conducted with InVision™ platform using a gene panel including cancer hotspots, entire coding regions and copy number variants (CNVs).

Study design

Mutation detection by InVision™ and ddPCR
Across both assays, 37 mutations were detected in 35 patients with InVision™ revealing substantially more diversity in mutations, with up to 8 individual mutations detected in a patient

There was 96.15% agreement for PIK3CA mutation detection between assays (Kappa 0.89, 95% CI 0.743 to 1.000), and 100% agreement for ESR1 mutations (Kappa 1.00, 95% CI 1.000 to 1.000)

Conclusions
This study demonstrates that ctDNA analysis using InVision™ and ddPCR have very high agreement for mutation detection and Allele Frequency quantification in patients with advanced breast cancer. There was high technical reproducibility in two independently processed blood samples, with Streck tubes presenting a robust alternative to immediate processing of samples. InVision™ had high clinical validity in HER2 amplification detection in this small cohort of patients.

Validation of HER2 amplification detected by InVision™
The sensitivity and specificity for HER2 amplification detection by InVision™ was 100% compared to tumor HER2 status determined with immunohistochemistry and/or FISH

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