Poster Number: 2743

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

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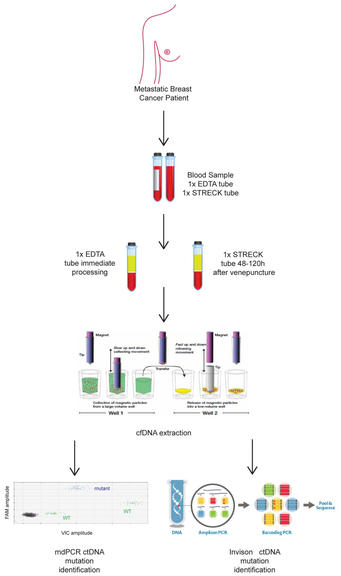
Background

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

Study design

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)



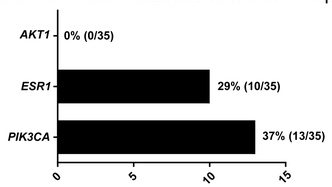
Clinicopathological characteristics of study patients

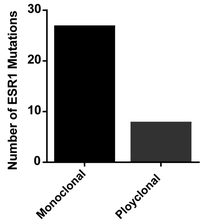
	n	%
n	35	
median age range	60 (33-79)	
Pathology		
IDC	20	57.1
ILC	4	11.4
Adenocarcinoma	5	14.3
Other	6	17.1
Histologic Grade		
1	0	0.0
2	11	31.4
3	11	31.4
N/A	13	37.1

	n	%
Receptor Status		
ER/PR+ HER2-	18	51.4
ER/PR- HER2+	8	22.9
ER/PR+ HER2+	1	2.9
TNBC	8	22.9
Nodal Status		
Negative	14	40.0
Positive	18	51.4
N/A	3	8.6

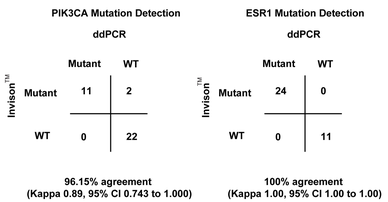
Mutation detection by InVision™ and ddPCR

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

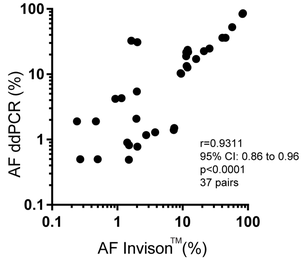




There was 96.15% agreement for PIK3CA mutation detection between assays (Kappa 0.89, 95% CI 0.743 to 1.000), and 100% agreement for ESR1 mutations (Kappa 1.00, 95% CI 1.000 to 1.000)



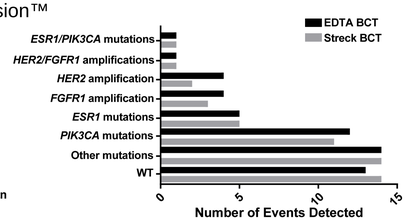
Correlation in AF between InVision™ and ddPCR

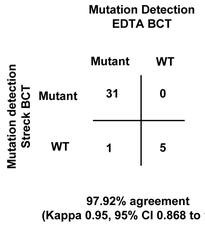


There was high correlation in mutation Allele Frequency (AF) between InVision™ and ddPCR (r=0.93, 95%CI 0.86 to 0.96, p<0.0001. Spearman correlation coefficient)

Performance of InVision™ in EDTA vs Streck BCT

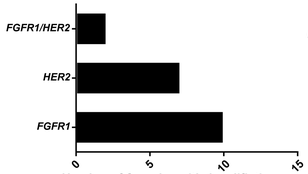
Individual event distribution, including mutations and amplifications, in ctDNA sequenced from immediately processed EDTA and delayed Streck BCT using InVision™

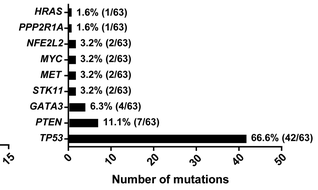




Comparison of immediate processing and Streck tubes revealed 97.92% agreement (Kappa 0.95, 95% CI 0.868 to 1.000) for mutation calling using InVision™

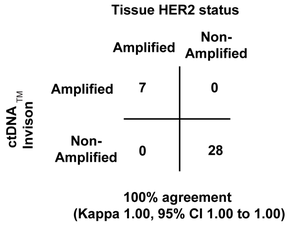
Amplifications and other mutations detected by InVision™





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



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

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