

Detection of low-frequency mutations in plasma ctDNA: performance assessment of a targeted ngs ivd assay in metastatic breast cancer

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Abstract

Background: Liquid biopsy has become clinically relevant in metastatic breast cancer (MBC) for assessing actionable biomarkers. Due to the low frequencies of tumor-derived mutations in plasma circulating tumor DNA (ctDNA), highly sensitive technologies are required for accurate mutation detection.

Objective: This study evaluated the analytical performance of a targeted NGS assay (Plasma-SeqSensei Breast Cancer IVD Kit (PQS), Sysmex), which detects VAFs as low as 0.06% or 6 mutant allele molecules (MM) per sample, in MBC patients.

Methods: Plasma samples were prospectively collected from n=10 unselected MBC patients (n=9, Hormone Receptor positive (HR+)/HER2-; n=1, Triple Negative MBC). Circulating free DNA (cfDNA) was extracted from 4mL of plasma and analyzed using the PQS assay, which targets AKT1, ERBB2, ESR1, KRAS, PIK3CA, and TP53, and sequenced on a NextSeq 550 (Illumina) platform. Data analysis was performed using the PQS IVD Software. In seven cases, ctDNA results were compared with matched prior plasma and/or tissue samples.

Results: Among the 10 plasma samples analyzed using the PQS assay, 5 were wild-type, while the others harbored mutations. Specifically, detected mutations were in PIK3CA (n=1 case), PIK3CA and TP53 (n=1 case), and TP53 alone (n=3 cases). VAFs ranged from 0.07% to 35.7%, corresponding to 6 to 2,113 MM per sample. In 7 cases, ctDNA results were compared with patient-matched tissue or prior liquid biopsy plasma samples, revealing an overall concordance of 71.4% (5/7) for genes covered by the PQS panel. The two discordant cases (28.6%) were HR+/HER2- MBC. In one case, a PIK3CA p.C420R mutation was detected in the tissue sample but not in plasma, likely due to low ctDNA levels or spatial heterogeneity. In another case, a TP53 p.R282W mutation was detected in plasma at a VAF of 0.58% (MM 23) but was not present in the matched tissue sample.

Conclusions: These results highlight the analytical performance of the PQS assay in detecting low-frequency variants in plasma and underscore the potential impact of temporal and spatial tumor heterogeneity between liquid and tissue-based analyses.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

Veracyte, Sysmex