Evaluating the complementarity of ngs and dpcr assays for ctdna-based esr1 mutation detection in hr+/her2- metastatic breast cancer

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Abstract

Background: ESR1 mutation testing in patients with hormone receptor-positive (HR+)/HER2-negative metastatic breast cancer (MBC) necessitates a liquid-first approach, relying on circulating tumor DNA (ctDNA) analysis. Given the subclonal nature of ESR1 mutations and the often low ctDNA abundance in plasma, highly sensitive analytical techniques, such as next-generation sequencing (NGS) and digital PCR (dPCR), are essential for accurate detection.

Objective: This study aimed to evaluate the complementarity of NGS and dPCR in a routine diagnostic workflow for ESR1 mutation testing.

Methods: Clinical and genomic data were retrospectively collected from 251 patients with HR+/HER2- (MBC) who underwent ESR1 ctDNA testing at the European Institute of Oncology (IEO) between November 2023 and February 2025. ESR1 mutation status was assessed using liquid biopsy (LB)-based NGS with the AVENIO ctDNA Expanded Kit, which provides full-gene ESR1 coverage with a variant allele frequency (VAF) detection threshold of ≥0.5%, and/or dPCR with the ddPLEX ESR1 Mutation Detection Kit, which targets hotspot mutations (E380Q, D538G, Y537S/N/C, L536R, and S463P) with an analytical sensitivity of 0.01%–0.025% VAF.

Results: Of the 241 successfully analyzed samples, 228 (94.6%) were assessed using NGS, 5 (2.1%) with dPCR, and 8 (3.3%) with both NGS and dPCR. Overall, 77 patients (32.0%) were ESR1mut, of whom 54 (70.1%) harbored a single ESR1 mutation, while 23 (29.9%) exhibited polyclonal ESR1 alterations. The most frequently detected mutations were Y537S/N/C (50.4%), D538G (30%), E380Q (8.5%), and L536H/P/R/K (4.3%). NGS also identified non-hotspot ESR1 mutations, including M472V, E542D, A67T, K268T, Q122*, L291P, S106C, and H6Y. The VAF of ESR1 mutations ranged from 0.12% to 45.8%. All 8 cases analyzed with both assays showed concordant ESR1 status.

Conclusions: NGS and dPCR are complementary for ESR1 testing in HR+/HER2- MBC. NGS enables comprehensive profiling including non-hotspot variants, while dPCR offers higher sensitivity for low-VAF variants and is preferable for targeted analyses. Their high concordance supports an integrated approach to optimize ESR1 testing in clinical practice.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.