ANALYTICAL CONCORDANCE OF NGS-BASED TESTS FOR ESR1 MUTATION DETECTION IN PLASMA

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BACKGROUND

Activating mutations in the *ESR1* gene represent a well-known mechanism of acquired resistance to aromatase inhibitors (AIs) in patients with hormone receptor-positive (HR+) metastatic breast cancer (mBC). Plasma-based liquid biopsy, through the analysis of circulating free DNA (cfDNA), provides a non-invasive and dynamic approach to detect *ESR1* mutations, enabling real-time monitoring of tumor evolution. This is especially relevant in the context of novel oral selective estrogen receptor degraders (SERDs), where *ESR1* mutation profiling will inform treatment selection and act as a biomarker of resistance and response. As these agents become part of routine clinical care, the ability to accurately detect and monitor *ESR1* mutations in plasma is critical.

As several next-generation sequencing (NGS)-based assays are commercially available, studies comparing their analytical concordance in detecting *ESR1* mutations in real-life plasma samples are needed.

OBJECTIVES

This study assessed the analytical concordance for the detection of *ESR1* mutations in cfDNA between two small commercially available amplicon-based NGS panels (Oncomine™ Precision Assay and Pillar Biosciences OncoReveal® Essential LBx) and a hybrid-capture based NGS test, VHIO360 which is a technology transfer of the Guardant360® assay to Vall d'Hebron Institutre of Oncology (VHIO).

MATERIALS AND METHODS

Clinically validated VHIO360 NGS assay was selected as the reference method for this study. In-house analytical validation with reference samples (SeraSeq), determined sensitivity at MAF ≥0.5% of 0.965 and <0.5% of 0.712.

100 plasma samples from appropriately consented mBC patients with known *ESR1* mutation genotypes established by VHIO360 testing during the VHIO Molecular Prescreening Program were selected for the study. The samples carried 64 pathogenic/likely pathogenic *ESR1* variants (59 corresponding to hotspot Y537S/N/C, D538G, and E380Q/K) with variant allelic frequencies (VAFs) ranging from 0.02 to 69% were tested in two molecular pathology labs with ThermoFisher and Pillar NGS assays (Fig 1). The analytical concordance to the reference VHIO360 assay was established in terms of agreements with the reference method (Tables 1 and 2). The degree of agreement correlated directly with to variant allelic frequencies of *ESR1* mutations (Fig.4).

RESULTS

Fig. 1. Study Overview

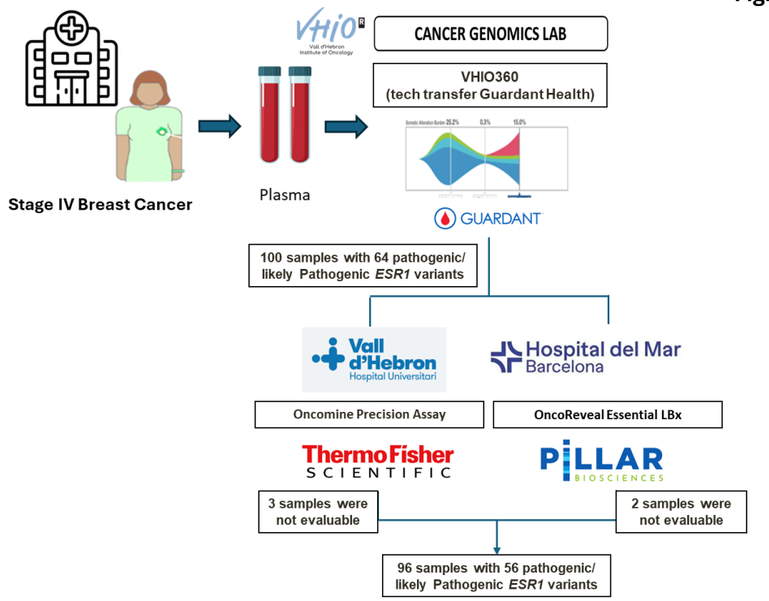


Fig. 2. Overall genotyping results. Venn diagram showing the pathogenic/ likely pathogenic reported variants detected by the 3 tests in *ESR1* gene.

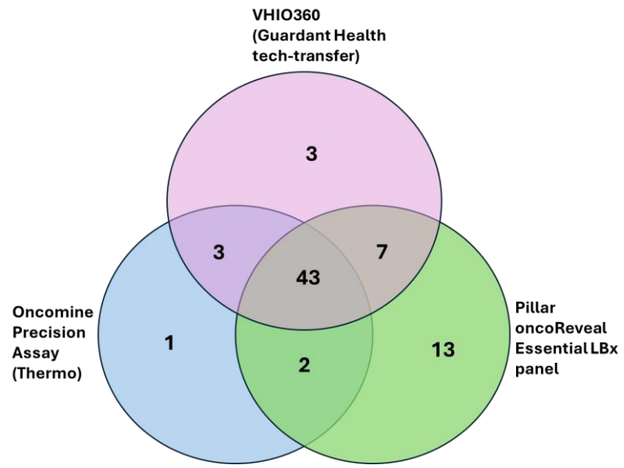


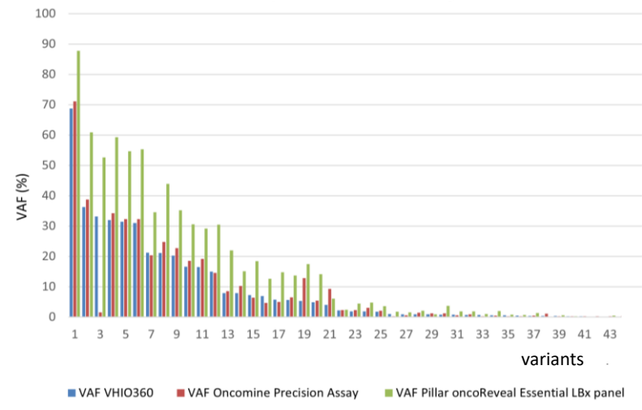
Table 1. Overall concordance rates between VHIO360 and Oncomine Precision Assay (Thermo Fisher) or OncoReveal Essential LBx (Pillar Biosciences) tests

ESR1 mutation concordance rates with VHIO360	
Oncomine Precision Assay	**Pillar oncoReveal Essential LBx panel**
82,1% (46/56)	89,23% (50/56)

Table 2. Detection rates of Oncomine Precision Assay (Thermo Fisher) or OncoReveal® Essential LBx (Pillar Biosciences) tests by *ESR1* VAF.

ESR1 mutation detection rates by variant allele fraction (VAF)		
% VAF	**Oncomine Precision Assay**	**Pillar oncoReveal Essential LBx panel**
≥0,5%	94,7% (36/38)	97,4% (37/38)
0,1-0,5 %	64,3% (9/14)	78,6% (11/14)
<0,1%	25% (1/4)	50% (2/4)

Fig. 4. *ESR1* gene pathogenic/ likely pathogenic VAFs of common reported variants by all three tests



CONCLUSIONS

- This study provides evidence with regards to the analytical performance of commonly used NGS-based assays for detecting *ESR1* mutations in plasma in comparison with a clinically validated method.
- Overall, relatively high concordance (82.1% and 89.2%) between VHIO360 and the Oncomine™ Precision Assay and Pillar Biosciences™ OncoReveal Essential LBx tests was established. More detailed analysis with bigger sample set will be reported elsewhere.
- As SERDs and other ESR1-targeted therapies become integrated into standard-of-care for HR+ metastatic breast cancer, accurate and validated mutation testing is essential in order to guide clinicians in selecting appropriate local testing approaches.