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ESR1 testing on FFPE samples from metastatic lesions in HR + /HER2- breast cancer after progression on CDK4/6 inhibitor therapy

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Abstract

Mutations in *ESR1* play a critical role in resistance to endocrine therapy (ET) in hormone receptor-positive (HR +)/HER2- metastatic breast cancer (MBC). Testing for *ESR1* mutations is essential for guiding treatment with novel oral selective estrogen receptor degraders (SERDs) like elacestrant or camizestrant. While most studies have utilized liquid biopsy (LB) for mutation detection, the role of formalin-fixed paraffin-embedded (FFPE) tissue biopsy in this context remains unclear. In this study, we analyzed a cohort of HR + /HER2- MBC patients who experienced resistance to ET and CDK4/6 inhibitors. Next-generation sequencing (NGS) was performed on FFPE biopsy samples obtained from metastatic sites at the time of disease progression. *ESR1* mutations were detected in 24 out of 38 patients (63.2%), with p.D538G identified in 10 patients (45.5%) and p.Y537S in 6 patients (27.2%) as the most frequent alterations. One patient exhibited dual *ESR1* mutations, and a recurrent *ESR1-CCDC170* gene fusion was identified, underscoring the diversity and potential interplay of genetic alterations driving resistance in HR + /HER2- MBC. Notably, lung metastases were significantly more common in ESR1 mutant cases (8/24, 33.3%) compared to wild-type cases (1/14, 7.1%), while liver metastases showed no difference between mutant (12/24, 50.0%) and wild-type groups (7/14, 50.0%). Co-mutations in actionable pathways, particularly *PIK3CA*, were observed in n = 10 *ESR1* mutant tumors (41.6%), highlighting their contribution to resistance mechanisms and posing significant challenges for treatment selection, as these alterations may necessitate combination therapies to effectively target multiple resistance pathways. This study presents new insights into the prevalence and clinical significance of *ESR1* mutations in HR + /HER2- MBC, highlighting the potential utility of FFPE biopsy samples as a viable alternative or complementary

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approach to LB for mutation detection, particularly in resource-limited settings where access to ctDNA analysis may be constrained.

Keywords *ESR1* mutations, Metastatic breast cancer (MBC), Endocrine therapy resistance, FFPE tissue biopsy, ctDNA, CDK4/6 inhibitors, SERDs

Introduction

Mutations in *ESR1*, the gene encoding for the estrogen receptor (ER) alpha, represent a major mechanism of resistance to endocrine therapy (ET) in patients with hormone receptor-positive (HR+)/HER2- breast cancers [1–3]. In the metastatic setting, molecular testing for *ESR1* mutational status is required for treatment selection with elacestrant, a novel oral selective estrogen receptor degrader (SERD) [4–6]. The approval of this drug was based on the findings of the EMERALD clinical trial, which showed that women with pre-treated *ESR1*–mutated (*ESR1*mut) HR+/HER2- metastatic breast cancer (MBC)—approximately 43% of cases—achieved significantly improved progression-free survival (PFS) with elacestrant compared to those treated with standard-of-care therapies [7]. Accordingly, guidelines now recommend routine testing for *ESR1* mutations upon HR+/HER2- breast cancer recurrence or progression after ET, whether administered alone or in combination with CDK4/6 inhibitors [8–10]. Other next-generation SERDs, such as camizestrant, which are selected for patients based on *ESR1* testing, are likely to enter clinical practice in the near future [11].

Since *ESR1* mutations in HR+/HER2- MBC are an acquired resistance mechanism, rarely present in the primary tumor, circulating tumor DNA (ctDNA) testing has been effectively employed in clinical studies and it is considered the optimal diagnostic strategy for novel SERDs treatment selection [12–14]. This is supported by the less invasive nature of liquid biopsy (LB) compared to tissue biopsy, its relatively high sensitivity, and its potential to address, at least in part, the challenges of tumoral heterogeneity [15–18]. However, in many healthcare environments, formalin-fixed paraffin-embedded (FFPE) tissue remains often easier to access than LB, especially in resource-limited settings where specialized logistics and infrastructure for processing and analyzing ctDNA may be unavailable [19].

Although FFPE biopsy samples from metastatic sites are routinely employed in clinical molecular diagnostics, their specific utility for ESR1 mutation detection remains largely underexplored. Liquid biopsy offers a non-invasive alternative; however, its sensitivity can be limited in patients with low tumor DNA shedding or bone-only metastases, where ctDNA levels may fall below the threshold for reliable detection [20–22]. In such cases, FFPE-derived DNA from metastatic tissue might represent a possible complementary source for *ESR1* mutation analysis.

This raises a pragmatic question: can FFPE tissue serve as a reliable adjunct to liquid biopsy in the molecular testing of ESR1? This study aims to address this gap by characterizing the *ESR1* mutational landscape through next-generation sequencing (NGS) on archival FFPE biopsy samples obtained from metastatic sites in HR+/HER2-negative MBC patients who have progressed on ET and CDK4/6 inhibitor therapies.

Materials and methods

Study design and data acquisition

The study was conducted in accordance with the Declaration of Helsinki and received approval by the Institutional Review Board (IRB) of the European Institute of Oncology (IEO) IRCCS, Milan, Italy (approval #UID3472); In compliance with the EU General Data Protection Regulation (GDPR), all information regarding the recruited patients were pseudo-anonymized [23]. The study characterized the *ESR1* mutational status in a retrospective cohort of patients with HR+/HER2- MBC who developed resistance to the combination of ET and CDK4/6 inhibitor therapy in the pre-elacestrant era (April 2019-November 2023). All analyses were performed on FFPE biopsy samples from metastatic sites collected at the time of disease progression following CDK4/6 inhibitor therapy. Molecular testing was performed using a targeted NGS approach (Supplementary Methods S1) at four referral Centers (Supplementary Figure S1): IEO; University of Naples Federico II, Naples, Italy (Federico II); Policlinico University Campus Bio-Medico, Rome, Italy (Campus); and Policlinico Umberto I, Rome, Italy (Policlinico). Adopted NGS assays are: the Oncomine Comprehensive Assay (OCA, ThermoFisher) v3 (IEO and Campus), the Oncomine Precision Assay (OPA, ThermoFisher) (Federico II), and the FoundationOne®CDx test (Foundation Medicine) (Policlinico), as summarized in Table 1.

Statistical analyses

Descriptive analyses were conducted to summarize the demographic and clinical characteristics of the patient cohort, as well as treatment patterns. Continuous

Table 1 Type of NGS assay used to test the FFPE metastatic samples according to the participating centers

Center (nr. of patients)	NGS Assay
IEO (22)	OCA
Policlinico (8)	FoundationOne®CDx
Campus (7)	OCA
Federico II (1)	OPA

IEO European Institute of Oncology, *Milan* Policlinico, Policlinico Umberto I, *Rome* Campus, Policlinico University Campus Bio-Medico, *Rome* Federico II, University of Naples Federico II, *Naples* OCA, Oncomine Comprehensive Assay v3 (ThermoFisher), *OPA* Oncomine Precision Assay (ThermoFisher)

variables were characterized using mean and/or median, with normality assumption assessed via the Shapiro–Wilk test. For non-normally distributed variables, the median and interquartile range (IQR) were reported. Categorical variables, including the site of visceral metastases and *ESR1* mutation status, were presented as frequencies and percentages. Comparative analysis using t-tests, Mann–Whitney U tests, or chi-square tests, where appropriate, was conducted to ensure the comparability of groups based on *ESR1* mutation status. A p-value less than 0.05 was considered statistically significant. All statistical analyses were conducted using Statistics for Data Analysis, formerly SPSS, (version 29.0.1.0). Detailed code and analysis scripts are available upon request to facilitate transparency and reproducibility.

Results

Clinicopathologic features of patients with *ESR1* mutation detected in the metastatic sites

A total of 38 patients with HR + /HER2- MBC were included in the analysis. The cohort had a median age of 51.5 years (range: 27–80 years), and a median PFS on CDK4/6 inhibitors of 15.70 months (range: 3.17–45.83 months). Inclusion was based on the availability of adequate residual FFPE tissue in our archives, defined as a minimum of five unstained Sects. (5 µm thick) with at least 10% estimated tumor cell content, as assessed by a pathologist. Most patients exhibited consistent ER expression (≥ 10% positive tumor cells) at diagnosis ($n = 36$; 94.7%) and were PgR-positive ($n = 29$; 76.3%). HER2 expression was negative in all cases, with $n = 24$ patients (63.2%) classified as HER2-low (IHC score 1 + or 2 + /ISH-negative). NGS revealed $n = 25$ *ESR1* mutations in $n = 24$ patients (63.2%), including one case with dual mutations in the gene. A significant difference in age distribution was observed between the *ESR1*mut and *ESR1*wt groups (median age: 47.0 vs. 54.5 years; $p = 0.04$), with *ESR1*mut patients being significantly younger. No substantial differences were identified in ER, PgR, or HER2 expression between *ESR1*mut and *ESR1*wt groups. Interestingly, none of the two patients with ER-low primary tumors showed *ESR1* mutations in their metastases post-ET progression. In our cohort, PFS on CDK4/6 inhibitors showed a broadly overlapping distribution between patients with *ESR1wt* and those with *ESR1*mut. Specifically, the *ESR1wt* group had a PFS range of 4.27 to 45.00 months, with a median of 16.87 months, while the *ESR1*mut group exhibited a range of 3.17 to 45.83 months and a median of 15.70 months. Although the median PFS was slightly shorter in the *ESR1*mut group, the difference was modest and not suggestive of a clinically meaningful impact. Given the substantial overlap in ranges and the limited difference in medians, no significant difference in PFS is evident between the two groups based on these descriptive statistics alone. Regarding the site of recurrence after ET, liver metastases were the most frequent biopsy sites, equally represented in both *ESR1*mut ($n = 12$; 50.0%) and *ESR1*wt ($n = 7$; 50.0%) groups. Lymph node recurrence was observed in $n = 3$ *ESR1*mut patients (12.5%) compared to $n = 1$ *ESR1*wt patient (7.1%), while contralateral breast recurrence occurred in $n = 2$ patients from each group (*ESR1*mut, 8.3%; *ESR1*wt, 14.3%). Considering the overall clinical history of the patients included in this study, lung recurrence was significantly more frequent in the *ESR1*mut group ($n = 8$; 33.3%) compared to the *ESR1*wt group ($n = 1$; 7.1%). Similarly, lymph node recurrence was higher in the *ESR1*mut group than in the *ESR1*wt group ($n = 16$; 66.7% vs. $n = 8$; 57.1%). These findings emphasize the heterogeneity between *ESR1*mut and *ESR1*wt HR + /HER2- MBC. The clinicopathologic features of the patients included in this study, according to *ESR1* status, are summarized in Table 2.

Frequency and type of *ESR1* alterations and co-mutations in other actionable genes

The majority of *ESR1* aberrations were point mutations ($n = 22$; 88.0%) and were predominantly located in exon 10 of the reference transcript (NM_001122742), as shown in Table 3.

The most common mutations were distributed as follows: p.D538G ($n = 10$; 45.5%), p.Y537S ($n = 6$; 27.2%), p.Y537N ($n = 2$; 9.0%), p.Y537C ($n = 1$; 4.5%), p.L536P ($n = 1$; 4.5%), p.E380Q ($n = 1$; 4.5%), and p.T140K ($n = 1$; 4.5%). Additionally, one V422del, one *ESR1-CCDC170* pathological gene fusion, and one *ESR1* amplification, were detected among the selected cases. The distribution of *ESR1* mutations according to the biopsy sites revealed that 50% of *ESR1* mutations were present in the metastatic deposits to the liver (Fig. 1).

In addition to *ESR1*, mutations in other cancer genes were detected, including $n = 8$ *PIK3CA* hotspot mutations identified in $n = 6$ patients, representing 15.8% of the entire cohort (Table 4). Additionally, mutations in *AKT1*,

Table 2 Clinicopathologic characteristics and sites of recurrence of HR+/HER2-MBC patients by *ESR1* mutation status

	ESR1mut (*n* = 24; 63.2%)	ESR1wt (*n* = 14; 36.8%)	Total (*n* = 38)	*p*-value
Age at diagnosis, range (median)				
	27–80 (47.0)	33–72 (54.5)	27–80 (51.5)	0.04
ER, n (%)				
Low	0	2 (14.3)	2 (5.3)	
Positive	24 (100)	12 (85.7)	36 (94.7)	
PgR, n (%)				0.59
Positive	19 (79.2)	10 (71.4)	29 (76.3)	
Negative	5 (20.8)	4 (28.6)	9 (23.7)	
HER2, n (%)				0.20
Low	17 (70.8)	7 (50.0)	24 (63.2)	
Negative	7 (29.2)	7 (50.0)	14 (36.8)	
Site of recurrence after disease progression (biopsy site), n (%)				0.99
Liver	12 (50.0)	7 (50.0)	19 (50.0)	
Contralateral breast	2 (8.3)	2 (14.3)	4 (10.5)	
Lymph node	3 (12.5)	1 (7.1)	4 (10.5)	
Lung	2 (8.3)	1 (7.1)	3 (7.9)	
Bone	2 (8.3)	1 (7.1)	3 (7.9)	
Other	3 (12.5)	2 (14.3)	5 (13.2)	
Sites of recurrence during the overall patients history, n (%)				0.71
Liver	12 (50.0)	8 (57.1)	20 (52.6)	
Contralateral breast	2 (8.3)	2 (14.3)	4 (10.5)	
Lymph node	16 (66.7)	8 (57.1)	24 (63.2)	
Lung	8 (33.3)	1 (7.1)	9 (23.7)	
Bone	15 (62.5)	9 (64.3)	24 (63.2)	
Other	5 (20.8)	3 (21.4)	8 (21.1)	

ER estrogen receptor, *PgR* progesterone receptor

Table 3 Type and genomic location of *ESR1* alterations detected in FFPE samples

	n (%)
Total number of *ESR1* alterations	25 (100)
Point mutations	22 (88.0)
p.T140K, c.419C > A (E3)	1 (4.5)
p.E380Q, c.1138G > C (E7)	1 (4.5)
p.L536P, c.1607 T > C (E10)	1 (4.5)
p.Y537N, c.1609 T > A (E10)	2 (9.0)
p.Y537S, c.1610A > C (E10)	6 (27.2)
p.Y537C, c.1610A > G (E10)	1 (4.5)
p.D538G, c.1613A > G (E10)	10 (45.5)
Indels	
V422del (E8)	1 (4.0)
Rearrangements	
ESR1-CCDC170 (E2-E10)	1 (4.0)
Amplifications	
CNV 5.98	1 (4.0)

MTOR, and/or *PTEN* were detected in n = 3 patients, accounting for 7.9% of the cases. Altogether, 41.6% of tumors (n = 10 patients) with *ESR1* mutations exhibited alterations in the PI3K-Akt-mTOR pathway. Beyond the PI3K pathway, the *ESR1*mut group also exhibited a range of other significant concurrent gene alterations, including *TP53, EGFR*, *MYC*, *AR*, *MDM4*, and *IGF1R*. Additional mutations were found in growth factor signaling pathway (*FGFR4* and *FGF19*), cell cycle progression regulation (*CCND1* and *CCND3*), key tumor suppressors (*RB1*), DNA damage response and repair mechanism (*CHEK2*, *MLH1*, *MSH2*, *STK11*, and *RAD50*).

Discussion

In this study, we presented previously unavailable data on rising *ESR1* mutations in metastatic deposits at the time of disease progression on endocrine therapy (ET) and CDK4/6 inhibitor therapy in HR+/HER2- breast cancer. Our findings demonstrate that FFPE samples from metastases can serve as a valuable tool for informing treatment decisions with novel SERDs, complementing

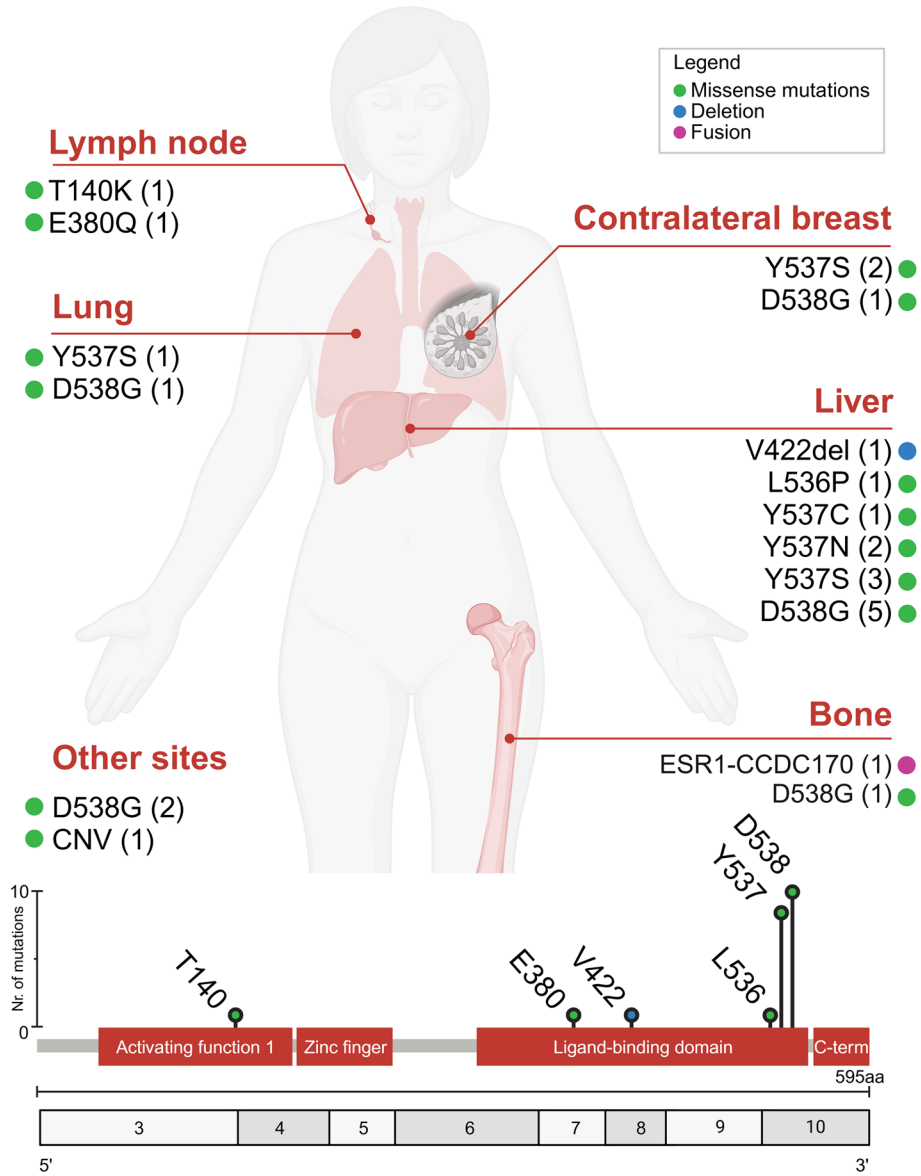

Fig. 1 *ESR1* mutation types and distribution across different recurrence sites after CDK4/6 inhibitor therapy. The figure highlights mutation types, their frequencies, the affected protein domains, and corresponding gene exons in patients with HR + /HER2- metastatic breast cancer, based on *ESR1* testing conducted on FFPE tissue samples collected in the recurrence site. Mutation types are color-coded for clarity, as indicated in the accompanying legend

ctDNA testing or providing an alternative in cases where LB is unavailable.

The identification of *ESR1* mutations in 63% of patients, as revealed by our NGS analyses, underscores a high prevalence of these alterations in this specific clinical setting. When comparing this frequency to other studies, the PADA-1 trial reported a prevalence of *ESR1* mutations in approximately 28% of patients with HR + / HER2- MBC treated with first-line aromatase inhibitors and CDK4/6 inhibitors, as detected through LB [24, 25]. Similarly, the EMERALD trial found *ESR1* mutations in 43% of pre-treated patients via ctDNA analysis [7, 26]. The relatively higher prevalence observed in our study may be attributed to differences in patient populations, detection methods, and sample sources, as our study focused on FFPE biopsies from metastatic sites, while the PADA-1 and EMERALD trials relied on ctDNA [27]. In the BOLERO-2 trial, conducted before the widespread use of CDK4/6 inhibitors, *ESR1* mutations were detected in 29% of patients using ddPCR [28,

Table 4 Frequency and type of *PIK3CA* mutations according to *ESR1* status

	ESR1mut (*n* = 24)	ESR1wt (*n* = 14)	Total (*n* = 38)
PIK3CA mutations, n (%)	8 (33.3)	8 (57.1)	16 (42.1)
p.V344M, c.1030G > A (E5)	1 (12.5)	0	1 (6.3)
p.E542K, c.1624G > A (E10)	0	2 (25.0)	2 (12.6)
p.E545K, c.1633G > A (E10)	2 (25.0)	1 (12.5)	3 (18.8)
p.E545Q, c.1633G > C (E10)	1 (12.5)	0	1 (6.3)
p.H556Y, c.1666C > T (E11)	0	1 (12.5)	1 (6.3)
p.E726K, c.2176G > A (E14)	1 (12.5)	0	1 (6.3)
p.H1047R, c.3140A > G (E21)	2 (25.0)	3 (37.5)	5 (31.3)
p.H1047L, c.3140A > T (E21)	1 (12.5)	1 (12.5)	2 (12.6)

A total of *n* = 8 *PIK3CA* mutations was detected in *n* = 6 *PIK3CA*-mutant cases in the ESR1mut group

29]. This lower prevalence may reflect differences in both the treatment landscape and detection methodology, as PCR-based sequencing has limited sensitivity compared to NGS in identifying diverse and low-frequency mutations [3, 30–35]. Notably, a secondary analysis of the trial revealed a threefold increase in mutation prevalence among patients who had progressed on first-line therapy for metastatic disease (33%) compared to those initiating first-line treatment (11%), underscoring the impact of prior treatment on mutational evolution [29]. Taken together, the variability of *ESR1* mutation rates in the literature could depend on the stage of disease, prior therapies, type of samples used for testing (FFPE vs. ctDNA), and the sensitivity of the detection method [2, 36–41].

Mutations such as p.Y537S and p.D538G were the most frequently identified in our cohort, aligning with previous studies [3, 5, 14, 39, 42–45]. These mutations seem to be associated with distinct biological and clinical characteristics [46]. For instance, p.Y537S demonstrates greater resistance to estrogen deprivation, tamoxifen, fulvestrant, and novel drugs like bazedoxifene and rintodestrant, compared to p.D538G, which underscores its role in treatment resistance [47–49]. Conversely, p.D538G is associated with enhanced metastatic potential, particularly to the liver, and has been implicated in the activation of Wnt signaling, a pathway not typically upregulated by p.Y537S [50, 51]. Our findings support these distinctions, as we identified five cases of p.D538G mutations specifically within liver metastases, highlighting the potential link between this mutation and liver tropism [14, 52–54]. Interestingly, *ESR1-CCDC170* gene fusion was identified in one case, a notable finding given its established role in ET resistance [55–57]. This fusion is particularly significant because it involves the ligand-binding domain (LBD) of *ESR1*, a region known to confer resistance to AI and to diminish the clinical efficacy of both selective estrogen receptor modulators (SERMs) and SERDs [40, 42, 58, 59]. The aberration leads to constitutive activation of ER signaling, independent of ligand binding, thereby driving resistance to ET [55]. Given the nature of this fusion and its recurrent presence in aggressive breast cancer subtypes, it is plausible that the *ESR1-CCDC170* alteration represents a constitutive event that may also be present in the primary tumor, preceding the selective pressure of therapy [60, 61]. Future studies investigating matched primary and metastatic tumor samples could clarify whether this fusion emerges de novo during progression or is an early, stable event driving the tumor's ET-resistant phenotype. One patient in our study exhibited dual *ESR1* mutations, underscoring intra-patient molecular heterogeneity in HR + /HER2- MBC. This highlights both the evolution of distinct subclones under the selective pressure of ET and the enhanced sensitivity of NGS in detecting rare or co-occurring alterations that may be overlooked by less comprehensive methods. Altogether, these findings emphasize the importance of profiling the full spectrum of *ESR1* mutations to better understand their functional implications and optimize therapeutic strategies.

Our study revealed clinically significant differences in metastatic patterns associated with *ESR1* mutation status. While the prevalence of liver metastases was similar between the *ESR1* mutant and wild-type groups (approximately 50%), lung metastases were significantly more common in *ESR1* mutant cases (33% vs. 7%). This contrasts with findings from a retrospective cohort of 3,388 HR + /HER2- MBC patients, which reported a slightly higher prevalence of liver metastases in *ESR1* mutant cases compared to wild-type [62]. The observed enrichment of lung metastases—particularly in cases harboring the *ESR1* D538G mutation—is intriguing and may reflect distinct biological behavior associated with specific *ESR1* mutant subclones. This specific mutation has been associated with altered expression of genes involved in epithelial-to-mesenchymal transition (EMT), cell motility, and extracellular matrix remodeling, which could

enhance metastatic potential [63]. These features may facilitate preferential dissemination to and colonization of the lung, a site known to support ER-positive breast cancer metastases through specific stromal and immune interactions [64–67]. While speculative, these findings warrant further investigation into potential organ-specific tropism driven by ESR1 mutations.

Interestingly, our NGS analysis identified co-occurring mutations, particularly in *PIK3CA*, in approximately 18% of cases, a rate higher than the 10–15% previously reported [68, 69]. Alterations in the PIK3CA/AKT1/PTEN pathway are well-established predictive biomarkers for response to targeted therapies such as alpelisib, capivasertib, and inavolisib [34, 70–74]. The high rate of PIK3CA co-mutations (41.6%) in ESR1mut tumors observed in our cohort has significant implications in terms of treatment prioritization and sequencing. When both mutations are present, clinicians must determine whether to first target the *ESR1* pathway with novel SERDs like elacestrant or the PI3K pathway with inhibitors such as alpelisib, capivasertib, or inavolisib. This decision often depends on several factors including the patient's prior treatment history, disease burden, symptomatology, and comorbidities. For instance, patients with aggressive visceral disease and both mutations might benefit from prioritizing PI3K pathway inhibition for its potentially more rapid response, while those with indolent progression might first receive ESR1-directed therapy [75]. Although combination approaches targeting both pathways simultaneously could theoretically address dual resistance mechanisms, clinical evidence supporting such strategies is currently limited, and potential increased toxicity remains a concern. The optimal sequencing or combination strategy remains undefined and represents an important area for future clinical investigation. These findings underscore the need for comprehensive molecular profiling to identify actionable co-mutations that may influence treatment selection and highlight the importance of developing evidence-based algorithms to guide sequential or combination therapy in patients with multiple resistance mechanisms.

Despite the insights provided, the present work has several limitations that warrant discussion. First, the retrospective design and reliance on archival FFPE biopsy samples may introduce selection bias, as only cases with sufficient tissue quality and quantity were included. This approach may not fully represent the broader HR+/HER2- MBC population. Second, our sample size was relatively small ($n=38$), which may limit the generalizability of the findings, particularly regarding the prevalence of co-mutations such as *PIK3CA* and *ESR1* or rare alterations like *ESR1-CCDC170* fusions. Larger studies are needed to validate the observed rates of these alterations and their potential clinical implications. Third, while NGS provides high sensitivity and broad mutation coverage, the study was unable to directly compare its performance with other diagnostic methods, such as ctDNA analysis via LB or ddPCR, which may have influenced the detection rates and the observed mutation spectrum. Additionally, the lack of paired primary and metastatic tumor samples precluded an analysis of how specific alterations emerge or evolve under therapeutic pressure. This limits our ability to distinguish between early constitutive events and acquired resistance mechanisms during treatment progression. Future studies integrating multi-omics approaches and longitudinal data are essential to address these limitations and refine therapeutic strategies for HR+/HER2-negative MBC.

Conclusions

This study provides now data on the prevalence and clinical relevance of *ESR1* mutations in HR+/HER2- MBC, emphasizing the potential of FFPE biopsy samples as an alternative or complementary tool to LB for mutation detection in resource-limited settings. NGS on FFPE tissue biopsies taken at the time of disease progression allowed us to identify a high frequency of *ESR1* mutations and co-occurring alterations, including PIK3CA mutations, which underscore the complexity of resistance mechanisms and the importance of comprehensive mutational profiling. The distinct metastatic patterns associated with *ESR1* mutations, such as the higher prevalence of lung metastases, and the identification of rare alterations like *ESR1-CCDC170* gene fusions and dual *ESR1* mutations, underscore the importance of incorporating comprehensive mutational profiling into routine clinical practice to guide more tailored and effective treatment strategies. Future prospective studies integrating matched primary and metastatic tumor samples and longitudinal analyses are essential to validate these observations and further inform therapeutic decisions in this evolving landscape.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13058-025-02020-x.

Supplementary Material 1.

Supplementary Material 2: Figure S1. Participating centers and technologies used for molecular testing by next-generation sequencing (NGS).

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natural language processing tool, was used for grammar checking. All content was reviewed and verified by the authors for accuracy and originality.

Authors' contributions
K.V. and G.C. wrote the main manuscript, and C.F. prepared the figures. E–G.R., C.C., and N.F. supervised the work. All authors provided samples and data, and critically reviewed the manuscript.

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Data availability
Sequence data that support the findings of this study is provided within the manuscript or supplementary information files. Raw sequencing data are available upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki and received approval by the Institutional Review Board (IRB) of the European Institute of Oncology (IEO) IRCCS, Milan, Italy (approval #UID3472); in compliance with the EU General Data Protection Regulation (GDPR), all information regarding the recruited patients were pseudo-anonymized. Informed consent was obtained from all participants prior to their inclusion in the study.

Competing interests
K.V. Has received honoraria for speaker bureau from Merck Sharp & Dohme (MSD), Roche, and AstraZeneca; G.Curs. from Veracyte; M.D-E from AstraZeneca, A.M. has received support from Menarini Group and served on the Speakers' Bureau for Roche and AstraZeneca. F.P. has received personal fees (as consultant and/or speaker bureau) from Menarini group and Roche. S.S. reported speaker fees from Novartis, Pfizer, Roche, Lilly, BMS, and MSD. Advisory role for AstraZeneca and Daiichi-Sankyo. C.D-A. reported grants from Daiichi Sankyo, Gilead, and Novartis; and personal fees from Pfizer, AstraZeneca, Eli Lilly, Novartis, and Roche outside the submitted work. G.T. reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer, Boehringer Ingelheim, Eli Lilly, BMS, GSK, Menarini, AstraZeneca, Amgen and Bayer, unrelated to the current work. U.M. has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientific, Eli Lilly, Diaceutics, GSK, Merck and AstraZeneca, Janssen, Diatech, Novartis and Hedera unrelated to the current work. G.P. has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Diaceutics, Merck, AstraZeneca, Novartis, Daiichi Sankyo, Exact Sciences, Sakura Finetek Europe B.V., Diatech Pharmacogenetics unrelated to the current work. A.B. has advisory and consulting roles for Roche, MSD, Novartis, Pfizer, Lilly, Amgen, BMS, Gilead, Sofos, Daichii, and AstraZeneca. G.V. reported personal fees from Roche, AstraZeneca, Daiichi Sankyo, Pfizer, Agilent, Eli Lilly, and Gilead. G.Curi. has received honoraria for speaker engagements from Roche, Seattle Genetics, Novartis, Lilly, Pfizer, Foundation Medicine, NanoString, Samsung, Celltrion, BMS, and MSD; honoraria for consultancy from Roche, Seattle Genetics, and NanoString; honoraria for participation in advisory boards from Roche, Lilly, Pfizer, Foundation Medicine, Samsung, Celltrion, and Mylan; honoraria for writing engagements from Novartis and BMS; and honoraria for participation in the Ellipsis Scientific Affairs Group. He has also received institutional research funding for conducting phase I and II clinical trials from Pfizer, Roche, Novartis, Sanofi, Celgene, Servier, Orion, AstraZeneca, Seattle Genetics, AbbVie, Tesaro, BMS, Merck Serono, Merck Sharp & Dohme, Janssen-Cilag, Philogen, Bayer, Medivation, and Medimmune. E.G-R. has received advisory fees, honoraria, travel accommodations/expenses, grants, and/or non-financial support from AstraZeneca, Exact Sciences, GSK, Illumina, MSD, Novartis, Roche, and Thermo Fisher Scientific. C.C. has participated in advisory or consultancy roles and speakers' bureau engagements for Eli Lilly, Pfizer, Novartis, Roche, AstraZeneca, MSD, Daiichi Sankyo, Gilead, and Seagen. N.F. has received honoraria for consulting, advisory role, speaker bureau, travel, and/or research grants from Merck Sharp & Dohme (MSD), Merck, Novartis, AstraZeneca, Roche, Menarini Group, Daiichi Sankyo, GlaxoSmithKline (GSK), Gilead, Sysmex, Genomic Health, Veracyte, Sakura, Leica Biosystems, Lilly, Pfizer, ThermoFisher, Abbvie. These companies had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and/or in the decision to publish the results. All other authors declare no potential conflicts of interest.

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