

Clinical value of circulating *ESR1* mutations for patients with metastatic breast cancer: a meta-analysis

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Background: The clinical implication of plasma *ESR1* mutations in the estrogen receptor (ER)-positive metastatic breast cancer (MBC) patients who had progressed after prior aromatase inhibitor (AI)-based therapy remains controversial. We conducted the first meta-analysis to investigate the prognostic significance and predictive role of plasma *ESR1* mutations in MBC patients with prior exposure to AI therapy.

Materials and methods: We searched PubMed, Embase, and Cochrane Library databases for eligible studies. Meta-analysis was conducted to calculate combined hazard ratios (HRs) with 95% CIs for progression-free survival (PFS) and overall survival (OS). Subgroup and sensitivity analyses were also performed.

Results: This study enrolled a total of 1,530 patients with ER-positive MBC cases from six articles, including 429 *ESR1* mutation carriers (28.04%). Meta-analysis demonstrated that plasma *ESR1* mutation carriers had significantly worse PFS (HR: 1.40, 95% CI: 1.17–1.66; $P < 0.0001$) and OS (HR: 1.65, 95% CI: 1.36–2.01; $P < 0.0001$) compared to wild-type *ESR1*. Subgroup analysis showed that plasma *ESR1* mutations were associated with shorter PFS after AI-based treatment, but were not significantly predictive of outcome on fulvestrant-containing therapy (HR: 1.26, 95% CI: 0.98–1.62; $P = 0.077$). As for different *ESR1* mutations, D538G mutation implied significantly worse PFS (HR: 1.50, 95% CI: 1.18–1.91; $P = 0.01$), while Y537S mutation was not correlated with PFS (HR: 1.65, 95% CI: 0.87–1.73; $P = 0.134$).

Conclusion: The meta-analysis indicated that plasma *ESR1* mutation assessment may have prognostic significance and clinical value in guiding further endocrine therapy choice in ER+ MBC patients who received prior AI therapy.

Keywords: *ESR1* mutations, breast carcinoma, cfDNA, ctDNA, meta analysis

Introduction

Approximately 75% of breast cancers are estrogen receptor (ER) positive, and the mainstay of treatment for this population is endocrine therapies such as selective ER modulators/downregulators, or estrogen deprivation by aromatase inhibitors (AIs).^{1,2} For patients with metastatic breast cancer (MBC), endocrine therapy is the preferred initial treatment, but unfortunately, almost all patients in this setting will develop endocrine resistance during treatment.^{3–5}

Although several mechanisms have been linked to endocrine resistance, no biomarker has reached wide clinical use.^{6,7} Recent studies have identified a set of mutations in the *ESR1* gene, which encodes ER α , from patients with endocrine-refractory MBC.⁸ Compared to primary breast cancers, *ESR1* mutations are more prevalent in MBCs,

particularly in those previously treated with AIs. According to studies using digital polymerase chain reaction techniques, ESR1 mutations were detected in 20%–55% of biopsies of ER-positive MBC patients.^{9–12} Most of these mutations are observed in the ligand-binding domain of the ESR1 gene, with D538G and Y537S being the most frequent ones.^{10,13,14} Recently, research has focused on detecting ESR1 mutations in “liquid biopsies” such as circulating cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA) as an alternative to metastatic tumor tissue, which enables easier sampling.^{15–18} Several studies that investigated the association of plasma ESR1 mutations with the outcomes of endocrine therapies have shown that detection of ESR1 mutations at baseline blood draw predicts a shorter progression-free survival (PFS) after treatment with AIs.^{19–23}

However, consensus has not been reached regarding the reliability of ESR1 mutations in cfDNA/ctDNA as predictive biomarkers due to some limitations with the current evidence.¹² First, most of these reports included a limited number of patients, and thus, inconsistent findings exist. Moreover, these studies also differ in many aspects, such as mutations assessed, drugs administered, methods to process plasma, and techniques to detect mutations. These drawbacks make it underpowered to analyze the differential effects of different ESR1 mutations and their predictive value for distinct therapeutic agents such as AIs or fulvestrant.

Considering the weakness of the individual studies, we carried out a meta-analysis to evaluate the impact of ESR1 mutation status in cfDNA or ctDNA on disease-free survival and overall survival (OS) in patients with ER-positive MBC. Subgroup analyses were performed to assess the clinical relevance of the two most frequent ESR1 mutations (Y537S and D538G) and to elucidate the predictive significance of ESR1 mutations on AI-based and fulvestrant-containing therapies.

Materials and methods

Publication search

The electronic databases of PubMed, Embase, and Cochrane Library were comprehensively searched for relevant studies between 1990 and 2017 using the following keywords and their combinations: “breast cancer” OR “breast neoplasm” OR “breast tumor”, “ctDNA” OR “cfDNA” OR “cell free DNA” OR “circulating” OR “plasma”, “ESR1” OR “Estrogen receptor α ” OR “ER α ”, and “therapy” OR “therapies”. Additionally, a manual search was performed to further

retrieve eligible studies using reference lists of the publications identified from the original search.

Criteria for inclusion and exclusion

Records identified from databases and reference lists were firstly screened by titles and abstracts and then further reviewed by full-text articles. Eligibility criteria were as follows: 1) studies enrolled patients with ER-positive MBC; 2) *ESR1* mutation status should be detected by cfDNA or ctDNA at baseline of certain endocrine therapy; and 3) the association between *ESR1* mutations and survival status was investigated.

Studies were excluded if they were non-English articles, reviews, commentaries, or case reports. Other exclusion criteria included: 1) articles not available of hazard ratio (HR) with 95% CI for PFS and/or OS; 2) lacking treatment information after baseline *ESR1* mutation analysis; and 3) duplicate reports from one study.

Quality assessment of studies

The quality of all relevant articles was evaluated independently by two authors (KZ and RH) using the Newcastle–Ottawa Scale (NOS) Quality Assessment Scale.²⁴ The NOS consists of three parts: selection, comparability, and outcome (cohort studies). We used total scores to assess the quality of eligible studies. Study with a score of 7 or higher was regarded as high quality.

Data extraction

Two investigators (KZ and RH) independently extracted the following data from the original studies: first author, publication year, *ESR1* mutations assessed, techniques used for *ESR1* mutation detection in ctDNA/cfDNA, number of patients enrolled, number of patients with plasma *ESR1* mutations, subsequent therapeutic regimens, and outcome data (HRs and 95% CIs for PFS/OS). If not reported by the articles, survival data were extracted from the Kaplan–Meier curves using the methods designed by Tierney et al.²⁵ Disagreements between two authors were resolved through common sense with the third investigator (SW).

Statistical analysis

Statistical analysis was carried out according to the guidelines of the Meta-Analysis of Observational Studies in Epidemiology group.²⁶ HRs and 95% CIs were performed for PFS and OS. I^2 and Q tests were performed to evaluate heterogeneity. $P < 0.1$ and $I^2 \geq 50\%$ indicated substantial

heterogeneity according to the Cochrane Handbook.²⁷ Fixed-effects or random-effects models were applied to estimate the HRs, depending on whether heterogeneity existed or not. Fixed-effects model was used if there was no evidence of heterogeneity between studies; otherwise, random-effects model was used. For all these analyses, P -value <0.05 was considered statistically significant. Funnel test, Begg's test, and Egger's test were carried out to assess publication bias. All statistical analyses were performed using STATA 12.0 software (StataCorp, College Station, TX, USA).

Results

Literature selection

The process of literature screening and study selection is shown in Figure 1. After preliminary online search, 195 citations were retrieved out of Embase, MEDLINE, and the Cochrane Library databases. After the deletion of duplicate results, 142 records remained. By reviewing the titles and abstracts, 119 records were excluded. Full texts of the remaining 23 articles were carefully reviewed and assessed, and 17 articles were further eliminated due to several reasons, leaving 5 eligible articles. In the report presented by Fribbens et al,²² data from two randomized studies (SoFEA and PALOMA3) were included, which were analyzed independently. Therefore, six eligible studies were finally enrolled in the meta-analysis.²⁸

Characteristics of included studies and patients

Characteristics of eligible studies are summarized in Table 1. The selected studies, published between 2015 and 2017, assessed the impact of *ESR1* mutations on the outcome to therapies in a total of 1,546 ER+ MBC patients. All the studies were retrospective, including four prospective-retrospective analyses using archived baseline plasma from randomized clinical trials. Sample types were cfDNA or ctDNA. There were 429 *ESR1* mutation carriers (28.04%). All patients received prior AIs. Subsequent therapeutic drugs were listed. The NOS scores of five included studies varied from 8 to 9 and the average NOS score was 8.5. An agreement between the two reviewers was 90% for study selection and 100% for quality assessment of these studies.

ESR1 mutations and PFS

Figure 2 shows the forest plot of the analysis about the relationship between circulating *ESR1* mutations and PFS. A total of six studies were subjected to this analysis. There was no obvious heterogeneity in the HRs for PFS among these studies ($P=0.400$, $I^2=4.2\%$). So, we used the fixed-effects model to calculate the pooled effect size. Our pooled analysis showed that plasma *ESR1* mutations were significantly associated with worse PFS compared with wild-type (WT) *ESR1* (HR: 1.40, 95% CI: 1.17–1.66; $P<0.0001$) in ER(+) MBC patients.

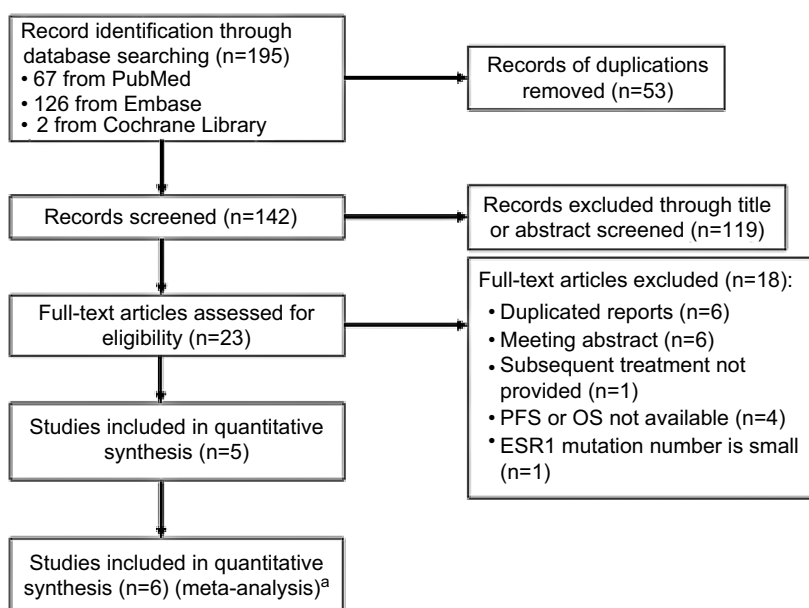


Figure 1 Flow diagram of literature review and study selection.

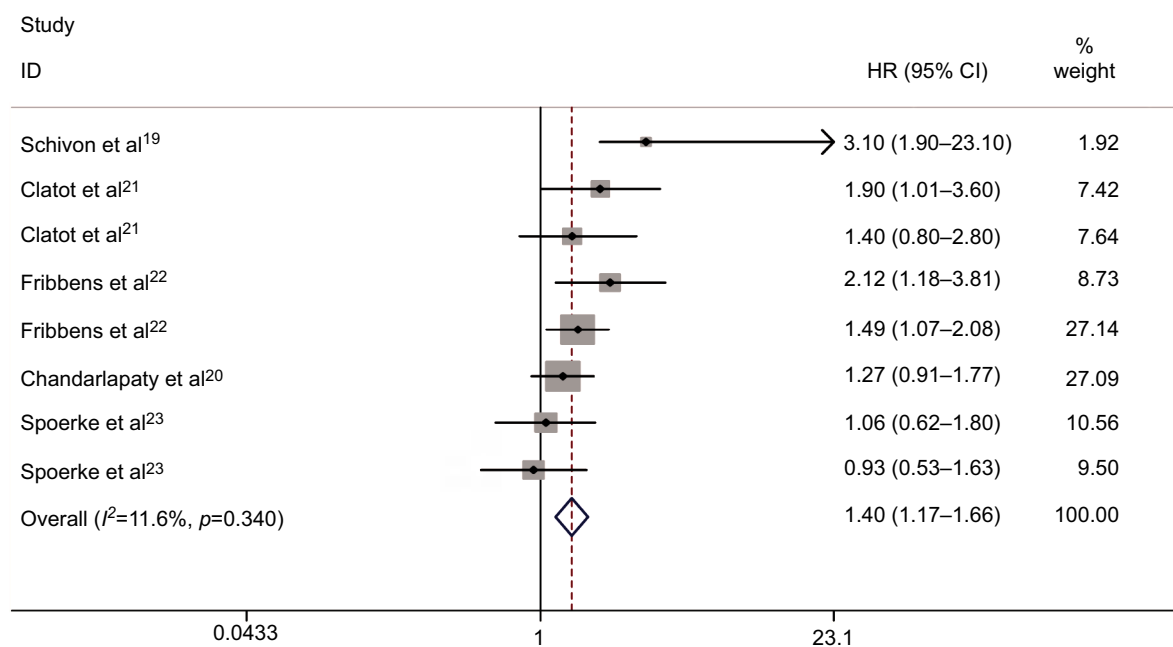
Notes: ³The report presented by Fribbens et al²² included data from two randomized studies (SoFEA and PALOMA3), which were analyzed independently. Therefore, six eligible studies were enrolled in the meta-analysis.

Abbreviations: OS, overall survival; PFS, progression-free survival.

Table 1 Characteristics of included studies

Author (trial)	Year	ESR1 mutations panel	No. of patients analyzed for ESR1	No. of ESR1 mutations-positive patients	Subsequent treatment	Sample type	Detection methods	Sampling time	Outcome	NOS
Schiavon et al ¹⁹	2016	D538G Y537S L536R Y537N Y537C	171	18	AI-based therapy	ctDNA	ddPCR	Baseline	PFS	9
Clatot et al ²¹	2016	D538G Y537S Y537N Y537C	144	44	AI based, chemotherapy, others	cfDNA	ddPCR	Baseline	PFS, OS	8
Fribbens et al ²² (SoFEA)	2016	D538G Y537S Y537N E380Q	161	63	Fulvestrant + anastrozole/placebo, exemestane	ctDNA	Multiple xddPCR	Baseline	PFS, OS	9
Fribbens et al ²² (PALOMA3)	2016	D538G Y537S Y537N E380Q	360	91	Fulvestrant + palbociclib/placebo	ctDNA	Multiple xddPCR	Baseline	PFS	9
Spoerke et al ²³ (FERGI)	2016	D538G Y537S E380Q Y537N Y537C P535H L536H L536P L536Q L536R	153	57	Fulvestrant + pictilisib/placebo	ctDNA	BEAMing	Baseline	PFS	9
Chandarlapaty et al ²⁰ (BOLERO-2)	2016	D538G Y537S	541	156	Exemestane + placebo/everolimus	cfDNA	ddPCR	Baseline	PFS, OS	9

Abbreviations: AI, aromatase inhibitor; ct, circulating tumor; cf, cell free; dd, droplet digital; NOS, Newcastle–Ottawa Scale; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival.

**Figure 2** Forest plot for PFS between plasma ESR1 mutation and wild-type ESR1.

Abbreviations: HR, hazard ratio; PFS, progression-free survival.

ESR1 mutations and OS

Figure 3 displays the forest plot of the analysis about OS. Five studies were subjected to the analysis. There was no evidence of heterogeneity in the HRs for OS among the individual trials ($P=0.971$, $I^2=0\%$). The combined HR with fixed-effects model for OS was 1.65 (95% CI: 1.36–2.01; $P<0.001$), indicating a prognostic association of *ESR1* mutations in patients with ER+ MBC.

Subgroup analyses

We conducted subgroup analyses according to the subsequent treatment for MBC patients after baseline sample collection (Table 2). The pooled HR was 1.51 (95% CI: 1.15–2.00; $P<0.001$) for PFS and 1.61 (95% CI: 1.29–2.00; $P<0.001$) for OS, suggesting that plasma *ESR1* mutation status was predictive of worse outcome on subsequent AI-based therapy. We then analyzed data from the three studies in which

fulvestrant-containing therapies were administered. It turned out that the *ESR1* mutations were not significantly correlated with PFS, with the pooled HR for PFS being 1.26 (95% CI: 0.98–1.62; $P=0.077$).

Subgroup analysis was conducted to further elucidate the impact of each *ESR1* amino acid mutation on treatment outcome, which is demonstrated in Table 3. Only two articles provided data on association between individual mutations and PFS, which were included in the subgroup analysis.^{20–22} The fixed-effects model was adopted to calculate the pooled HRs about *D538G* mutation due to absence of heterogeneity ($I^2=0.0\%$, $P=0.605$). *D538G* mutation suggested significantly worse PFS (HR: 1.50, 95% CI: 1.18–1.91; $P=0.01$) compared with WT *ESR1* in MBC patients receiving endocrine therapy. However, for *Y537S* mutation analysis, obvious heterogeneity was detected ($I^2=83.7\%$, $P=0.002$). Our result indicated that *Y537S* mutation was not correlated with PFS (HR: 1.65, 95% CI: 0.87–1.73; $P=0.134$).

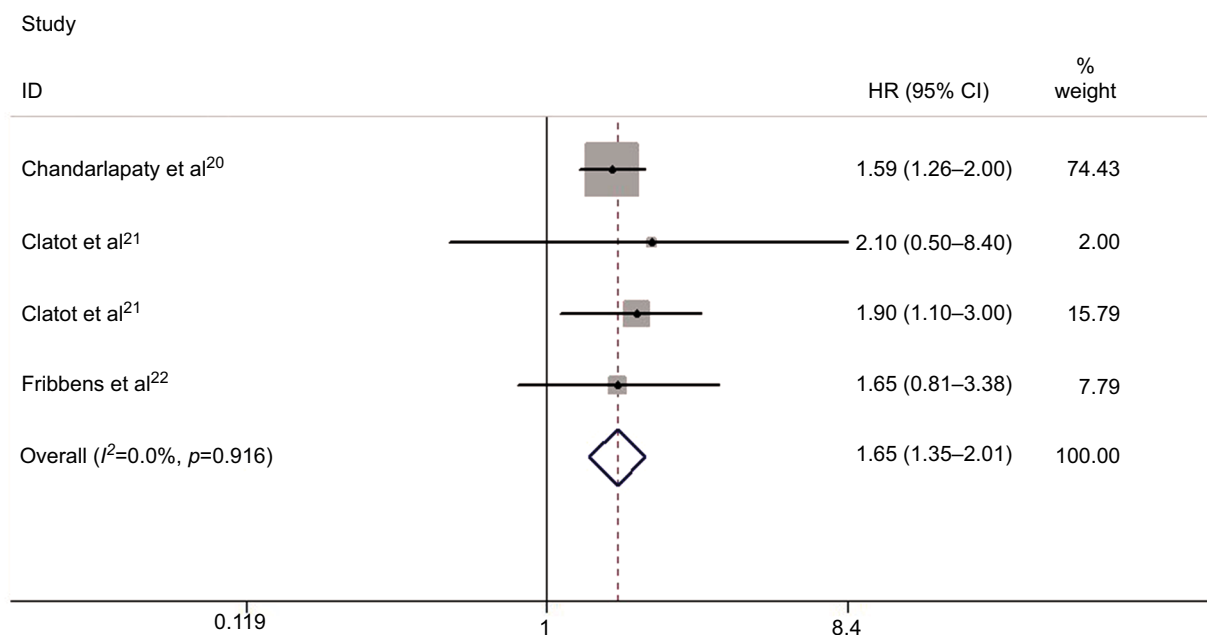


Figure 3 Forest plot for OS between plasma *ESR1* mutation and wild-type *ESR1*.
Abbreviations: HR, hazard ratio; OS, overall survival.

Table 2 Results of subgroup analyses for effects of subsequent lines of treatment on PFS or OS

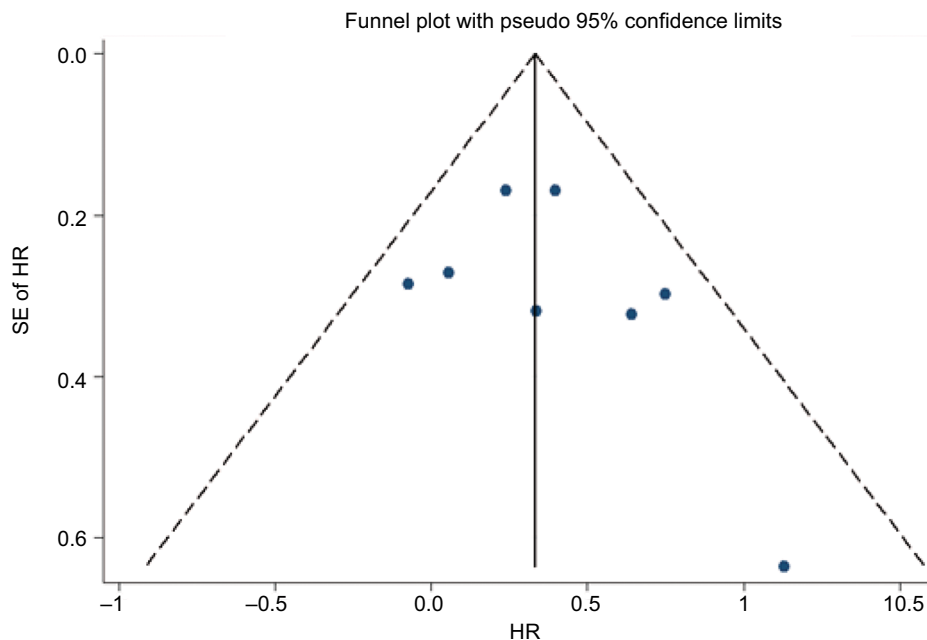
Subsequent lines of treatment	PFS			OS		
	df	HR (95% CI)	Test for heterogeneity (I^2)	df	HR (95% CI)	Test for heterogeneity (I^2)
AI-based therapy	3	1.51 (1.15–2.00)	26.7%	2	1.61 (1.29–2.00)	0.0%
Fulvestrant-containing therapy	2	1.26 (0.98–1.62)	20.1%	-----		

Abbreviations: AI, aromatase inhibitor; df, degrees of freedom; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

Table 3 Results of subgroup analyses for effects of *ESR1* mutation on PFS

<i>ESR1</i> mutation	<i>ESR1</i> mutation numbers	df	Model	HR (95% CI)	Test for heterogeneity (I^2)
D538G vs WT <i>ESR1</i>	121	2	Fixed	1.50 (1.18–1.91)	0.0%
Y537S vs WT <i>ESR1</i>	61	2	Random	1.65 (0.87–1.73)	83.7%

Abbreviations: df, degrees of freedom; HR, hazard ratio; PFS, progression-free survival; WT, wild type.

**Figure 4** Funnel plot to detect publication bias for PFS in the plasma *ESR1* mutations vs wild-type *ESR1*.

Abbreviations: PFS, progression-free survival; SE, standard error; HR, hazard ratio.

Publication bias and sensitivity analysis

Funnel plot, Begg's test, and Egger's test were performed to assess the publication bias of the included studies. There was no evidence of publication bias according to funnel plot (Figure 4), Egger's test ($P=0.357$), and Begg's test (0.386) for PFS. Similarly, publication bias was not significant for OS studies according to Egger's test ($P=0.255$) and Begg's test ($P=0.734$). Each study was sequentially omitted to evaluate whether any single study had a significant impact on the pooled OS or PFS. Our results showed that the pooled HRs were not influenced by any individual study.

Discussion

This meta-analysis evaluated the association between plasma *ESR1* mutations and survival of MBC patients. Our results showed that plasma *ESR1* mutations represent a worse prognostic factor in patients with MBC who received subsequent AI therapy. The combined HRs were 1.40 (95% CI: 1.18–1.67; $P<0.0001$) for disease-free survival and 1.66 (95% CI: 1.36–2.01; $P<0.0001$) for OS between mutated *ESR1* and WT *ESR1* in advanced ER-positive MBC after treatment

containing endocrine agents, suggesting that breast cancer with and without *ESR1* mutations are distinct subtypes that respond differently to therapies.

For patients who failed prior AI therapy, PFS after AI rechallenge was significantly decreased in those harboring *ESR1* mutations (HR: 1.51, 95% CI: 1.15–2.00; $P<0.001$). But in the fulvestrant treatment subgroup, the pooled HR (1.26, 95% CI: 0.98–1.62; $P=0.077$) did not significantly favor those with a WT *ESR1*, suggesting that *ESR1* mutations may not be correlated with innate or acquired fulvestrant resistance. This was consistent with previous in vitro studies that reported growth inhibition with fulvestrant in *ESR1* mutant cell lines.²⁸ These findings support the choice of fulvestrant over AI in treating MBC patients who had progressed after AI therapy and highlight the clinical implication of using plasma *ESR1* mutation testing to guide further endocrine-based therapy.

Detecting *ESR1* mutations in cfDNA/ctDNA has several obvious advantages over metastatic biopsies. Several studies have suggested that cfDNA or ctDNA sometimes reveal additional mutations compared with matching metastatic

tumor biopsies due to tumor heterogeneity.^{15–18} This indicates that cfDNA/ctDNA may be more representative of the whole tumor landscape. In addition, serum samples can be easily obtained in a noninvasive way for multiple times, enabling assessment of mutations not only at baseline, but also sequentially during therapy. A rise in *ESR1* mutations during treatment may indicate disease progression as a result of evolution through selective AI treatment. Upon treatment with fulvestrant, if resistant *ESR1* mutant clones decay, this could possibly allow rechallenges with AIs in this subset of patients.

Another major question is whether the various *ESR1* mutations lead to distinctive phenotypes; the D538G mutation implied shorter PFS compared to WT *ESR1* (HR: 1.50, 95% CI: 1.18–1.91; $P=0.01$) for breast cancer patients, but the Y537S mutation did not relate to shorter PFS (HR: 1.65, 95% CI: 0.87–1.73; $P=0.134$). This subgroup analysis may provide clinicians a precise guideline for those patients who have different *ESR1* mutations. However, several basic studies have shown that Y537S mutation represents the strongest ER activity under an estrogen-deprived condition, which is inconsistent with these findings.^{11,24} As for the Y537S mutation analysis, obvious statistical heterogeneity was detected ($I^2=83.7\%$), and in these two studies for Y537S mutations, there might be obvious heterogeneity between patients in the two different clinical studies.^{20,22} Moreover, owing to the limited number of articles, the relationship between different *ESR1* mutations and the prognosis of breast cancer cannot be very clearly elucidated. Functions of other alterations, such as E380Q, Y537N, Y537C, and L536R in plasma DNA, have not been addressed in the current studies. Thus, future studies evaluating all ligand-binding domain mutations in *ESR1* and their potential distinctive effects are of interest.

As we all know, *ERα* is an important gene for ER+ breast cancer, and gene signatures-derived cancer hallmarks are good prognostic biomarkers. Wang et al³³ created an algorithm to identify prognostic gene signature sets for ER-negative and -positive breast cancers. Also, eTumorType could be set aside for tumor diagnosis based upon the copy number variations of captured CTCs or cfDNAs in blood samples. The molecular mechanism of *PIK3CA* mutations in luminal A patients can participate in a positive regulatory loop (*PDGF-D/FLT1/SHC1*), and moreover, the regulatory loop has a potential power to predict the survival of luminal A patients with *PIK3CA* mutated. The next step may use these algorithms to identify prognostic gene signature for ER+ subtype. A cancer hallmark network framework for modeling

genome sequencing data forecast clinical phenotypes and cancer progression to better design patient treatment.^{29–33}

Our results are promising to a certain extent, but several limitations of this meta-analysis still need to be discussed. First, the number of enrolled studies was limited and the sample size was relatively small, which might not have provided sufficient power of test to evaluate the associations between the plasma *ESR1* mutations and clinical outcome in ER+ MBC patients. Second, our meta-analysis is based on data from published articles, so publication bias and selection bias may exist. Third, these studies were retrospectively conducted, which used different patient selection criteria and involved various treatments. Finally, some data were extracted from the survival curves and, thus, might not be accurate.

In conclusion, our results suggest that plasma *ESR1* mutation analysis may have prognostic value and clinical implication in directing further endocrine therapy choice in ER+ MBC patients who failed prior treatment with AIs. Patients with *ESR1* mutations and WT *ESR1* seem to be different subtypes of advanced ER-positive breast cancer. Future clinical trials may use plasma DNA analysis to guide treatment selection according to *ESR1* mutation status.

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Disclosure

The authors report no conflicts of interest in this work.

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