The clinical impact of plasma estrogen receptor-1 mutation in patients with metastatic breast cancer: A meta-analysis

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Abstract

Background. The relevance of the discovered plasma *ESR1* mutations in positive metastatic breast cancer (BC) patients who had progressing disease after aromatase inhibitor (AI)-based therapy is still being debated.

Objectives. We conducted this meta-analysis to explore the prognostic and predictive role of plasma *ESR1* mutations in patients with progressive BC who have previously received AI therapy.

Materials and methods. We searched for relevant studies in the PubMed, Embase and Cochrane Library databases to be included in the meta-analysis. This study was performed to compute combined hazard ratios (HRs) with 95% confidence intervals (95% CIs) for the progression-free survival (PFS) rate and overall survival (OS) rate. Subgroup and sensitivity analyses were also performed. The heterogeneity between studies was evaluated using the I^2 statistic.

Results. In this meta-analysis, a total of 1,844 patients with metastatic BC and positive for estrogen receptors (ERs) were enrolled from 8 articles. The analysis revealed that patients with circulating *ESR1* mutations had significantly worse PFS (HR: 1.34; 95% CI: 1.17–1.55; $p < 0.001$) and OS (HR: 1.59; 95% CI: 1.31–1.92; $p < 0.001$) compared to wild-type *ESR1* patients. Subgroup analysis showed that the types of plasma *ESR1* mutations were associated with differences in the prognosis of metastatic BC. The D538G mutation showed a statistically significant lower PFS ($p = 0.03$), while the Y537S mutation was not significantly correlated with PFS ($p = 0.354$).

Conclusions. According to the findings of this meta-analysis, the assessment for plasma *ESR1* mutations may provide prognostic and clinical guidance regarding subsequent endocrine therapy decisions for ER-positive, metastatic BC patients who had received prior therapy with AIs.

Key words: meta-analysis, metastatic breast cancer, aromatase inhibitors, *ESR1* mutations, prognostic role

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Background

Breast cancer (BC) is among the most common tumors, with about 75% of BC cases being positive for estrogen receptors (ERs).[1,2] The cornerstone of treatment are endocrine therapies such as ER modulators or aromatase inhibitors (AIs).[3] The preferred initial treatment for metastatic breast cancer (MBC) is endocrine therapy; however, most patients are at risk for endocrine resistance during treatment.[4,5]

Several mechanisms and biomarkers have been associated with endocrine resistance, but the clinical application of these mechanisms has not yet been reached.[6] One crucial factor in their resistance is a mutation occurring in the binding domain of estrogen receptor 1 (ESR1) encoding ER-α.[7] In vitro investigations have provided evidence that the *ESR1* mutation gives rise to an ER that is constantly active, regardless of ligand binding. This constitutive activation of the ER leads to increased cell proliferation and reduced responsiveness to endocrine therapies.[8] This particular mutation has been the subject of extensive research in the past 10 years, with a focus on understanding its biochemical and molecular impacts, as well as its significance in determining suitable treatment options and identifying potential weaknesses that can be targeted therapeutically.[9]

A set of *ESR1* gene mutations has been identified in previous studies from patients with metastatic BCs. The prevalence of *ESR1* mutations is much higher in metastatic BC compared to primary cancers, especially in patients previously treated with AIs.[10,11]

The incidence of *ESR1* mutations among patients with MBC who have undergone AI therapy ranges from around 20% to 40%, with variations observed depending on the specific areas of metastatic disease.[12] Estrogen receptor 1 mutations were detected in about 55% of biopsies from ER-positive metastatic BC patients.[13] About 40% of MBCs acquire the *ESR1* mutations, which confers resistance to AI therapy in MBC.[14]

The most common mutations found in several investigations are the D538G and Y537S variants. The E380Q mutation is the 3rd most prevalent identified mutation.[15] Recently, most studies have focused on detecting *ESR1* gene mutations in circulating tumor DNA (ctDNA) for easier sampling as an alternative to tumor tissue biopsies.[16] Several clinical studies have provided evidence that *ESR1* mutations in the plasma are associated with a decreased progression-free survival (PFS) rate following AI treatment.[17–19] However, the utility and reliability of *ESR1* mutations in the plasma as a predictive tool for BC prognosis is still controversial due to the limitations in the current evidence. These limitations include a wide discrepancy in mutations assessed, drugs used, plasma processing methods, and techniques. Moreover, most studies included a limited number of patients, resulting in inconsistent findings. The aforementioned limitations render them insufficient in their capacity to effectively assess the varying impacts of various *ESR1* mutations and their utility in the prediction of efficacy of a specific treatment, such as AIs or fulvestrant.

Objectives

Therefore, the present meta-analysis aimed to assess the clinical utility of *ESR1* mutations in ctDNA and its impact on PFS and overall survival (OS) among MBC patients with ER-positive BC. We also carried out subgroup analysis to evaluate the relevance of the most frequent types of *ESR1* mutations, including D538G and Y537S, and their predictive significance in therapies based on AI regimens and fulvestrant.

Materials and methods

The current meta-analysis study was conducted based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.[20]

Search strategy and study selection

Observational, retrospective or prospective studies reporting the effect of *ESR1* mutations on the therapeutic outcomes of MBC patients with positive ER BC were included. Only clinical trials conducted on patients, regardless of the language used, were eligible for the analysis. The size or follow-up duration of the study did not limit the inclusion. At least 1 of the outcomes, including PFS and OS rates, had to be measured in the eligible studies. Commentaries, review articles and irrelevant studies were excluded.

Data sources and identification

First, we systematically searched the different electronic databases, including Embase, Cochrane Library and PubMed, until August 2022. The terms adapted and applied for searching each electronic database included BC, breast neoplasm, tumor, ctDNA, cell-free DNA (cfDNA), plasma, ESR1, ER, and therapy, as summarized in Table 1. No restrictions were applied regarding the location, design or language of the study. After the initial examination of the titles and abstracts, we gathered all eligible publications using EndNote software (Clarivate Plc, London, UK) into a single file to omit duplications. Studies that did not report the impact of *ESR1* mutations on PFS or OS among MBC patients were excluded. The retrieved publications were investigated for relevant outcome data. Publications with missing data were excluded.

Screening

A pre-designed form was utilized to summarize main features and properties of the studies. The main

Table 1. Search strategy for each database

Database	Search strategy
PubMed	#1 "breast cancer"[MeSH Terms] OR "breast neoplasm"[All Fields] OR "ESR1 mutation"[All Fields] OR "breast metastasis"[All Fields] #2 "aromatase inhibitor"[MeSH Terms] OR "PFS"[All Fields] OR "OS"[All Fields] OR "progression-free survival"[All Fields] OR "overall survival"[All Fields] OR "endocrine therapy"[All Fields] (word variations have been searched) #3 #1 AND #2
Embase	#1 'breast cancer'/exp OR 'breast neoplasm'/exp OR 'ESR1 mutation'/exp OR 'breast metastasis'/exp #2 'aromatase inhibitor'/exp OR 'PFS'/exp OR 'OS'/exp OR 'progression-free survival'/exp OR 'overall survival'/exp OR 'endocrine therapy'/exp (word variations have been searched) #3 #1 AND #2
Cochrane Library	#1 (breast cancer):ti,ab,kw OR (breast neoplasm):ti,ab,kw OR (ESR1 mutation):ti,ab,kw OR (breast metastasis):ti,ab,kw #2 (aromatase inhibitors):ti,ab,kw OR (PFS):ti,ab,kw OR (OS):ti,ab,kw OR (progression-free survival):ti,ab,kw OR (overall survival):ti,ab,kw OR (endocrine therapy):ti,ab,kw (word variations have been searched) #3 #1 AND #2

parameters included the surname of the first author, study timeframe, region, year of publication, age, the assessed *ESR1* mutation types, study techniques, subject number, therapeutic regimens, and outcome data such as hazard ratios (HRs) of PFS and OS. In cases of missing survival data, a Kaplan–Meier curve was used instead to extract data using the method described by Tierney et al.[21] A unique identification number was used for each citation. In case of a study's suitability for analysis, according to the applied inclusion criteria, data were retrieved by 2 of the authors individually. In cases of discrepancy, the corresponding author was consulted for the final decision. Each study was appraised by 2 authors who examined the procedural quality of the relevant trials individually.

The risk of bias

For the study's procedural quality evaluation and risk of bias assessment, the Cochrane risk-of-bias tool was implemented for randomized trials v. 2 (RoB 2).[22] The quality of selected publications was evaluated in duplicates, and discrepancies were cleared by consulting the corresponding author. The bias evaluation criteria included the assignment of the study to one of the following categories: the complete fulfillment of quality standards warranted the study classification to the low risk of bias category, partial fulfillment of the quality requirements (1 or more missing or not adequately clarified) warranted the publication categorization as having a moderate risk of bias, and the high risk of bias category applied to publications which failed to meet the quality standards. Reassessment of the original publication was applied in cases of any inconsistencies.

Eligibility

The main findings focused on the relation of *ESR1* gene mutations in BC patients with metastasis. A summary was created based on the presence of outcome data regarding PFS or OS. The identified records were screened initially based on their titles and abstract, and then the full text was reviewed. We conducted a comprehensive search to identify all available and relevant studies to identify the latest developments and limitations in the existing literature. Well-designed randomized controlled or comparative research studies, either prospective or retrospective, which enrolled patients with positive ER MBC in whom procedure of intervention included the detection of *ESR1* gene mutations by ctDNA or cfDNA at baseline of endocrine therapy, and which had adequately described data to estimate the overall pooled effect size of survival status association with *ESR1* mutations were included in this meta-analysis. Published case reports, abstracts, editorials, review articles, animal experiments, commentaries, studies with missing duplicate reports or incomplete data, studies lacking therapy information at baseline analysis of *ESR1* mutations, and research studies with irrelevant objectives were excluded from this meta-analysis.

Sensitivity analysis and subgroup assessment

Sensitivity analyses were applied only to studies that showed a high risk of bias or high heterogeneity to evaluate the impact on research findings.

Statistical analyses

Pooled HRs, 95% confidence interval range (95% CI), and p-values were estimated. We used Review Manager (RevMan v. 5.3; Nordic Cochrane Centre, Copenhagen, Denmark) and STATA v. 10.0 (StataCorp LLC, College Station, USA) for data analysis and visualization. The data were presented in forest plots. For computing statistical heterogeneity, we used the I^2 index, which was valued up to 100%.[17] The I^2 value of about 0% denoted no heterogeneity, while I^2 values of 25%, 50% and 75% denoted low, moderate and high heterogeneity, respectively.[23,24] The discrepancy between the included studies regarding eligibility criteria, population characteristics, potential bias, chemotherapeutic regimens, sample type applied, and study interventional arms was evaluated to determine the appropriate model

to employ in our study, whether it be a fixed-effect model or a random-effects model. The random-effects model was employed for all analyses based on the evaluation of disparities. For subgroup analyses, we stratified the outcomes per result category. We quantitatively assessed publication bias by Egger's regression,[25] Begg's rank correlation tests[26] and visual inspection of Begg's funnel plots. The entire estimated p-values were two-tailed. A p-value for differences amongst comparisons of < 0.05 denoted a statistically significant difference.

Results

The main characteristics of the included studies

The search of electronic databases resulted in 214 publications. After the search was restricted to clinical trials, 142 of them were retrieved. The removal of irrelevant studies and duplicates yielded 8 relevant articles for inclusion and analysis. Two randomized clinical trials were presented in a report by Fribbens et al.[27] (SoFEA and PALOMA3) and were independently analyzed. Figure 1 summarizes the search strategy for the databases and the selection for meta-analysis.

Table 2 illustrates 8 randomized trials[27–34] published between 2016 and 2021. All trials were retrospective, including prospective-retrospective studies (n = 5) using the baseline archived plasma from randomized trials. The sample size in the eligible studies ranged from 16 to 541 patients, with a total of 1,844 patients enrolled. All participants received prior therapy with AIs with different subsequent treatment regimens, as listed in Table 2. The risk of bias in the selected studies was assessed in accordance with the Cochrane Collaboration tool. Study selection was based on 95% agreement between the 2 authors, and they both agreed 100% on the quality rate of the selected studies.

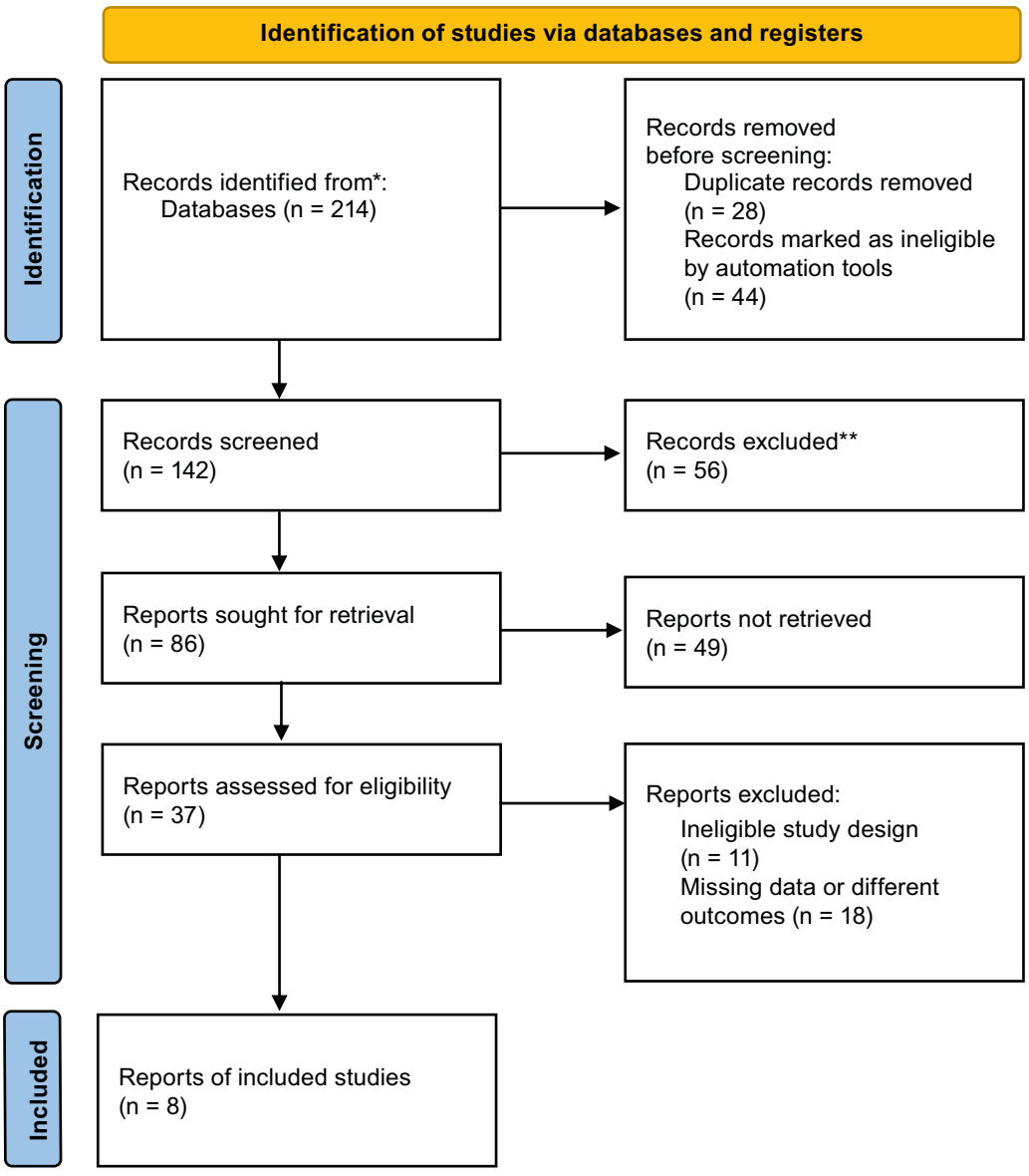

Fig. 1. Flowchart of the study search strategy

Table 2. Characteristics of the selected studies

Outcome(s) of interest measured	Subsequent therapy	Sample type	Sample size	Study ID, year
PFS, OS PFS	fulvestrant and anastrozole, exemestane fulvestrant and palbociclib/placebo	ctDNA	161 360	Fribbens et al., 2016[27] SoFEA PALOMA
PFS, OS	AI-based therapy	cfDNA	144	Clatot et al., 2016[28]
PFS	AI-based therapy	ctDNA	171	Schiavon et al., 2016[29]
PFS	fulvestrant and pictilisib	ctDNA	153	Spoerke et al., 2016[30]
PFS, OS	exemestane and placebo/everolimus	cfDNA	541	Chandarlapaty et al., 2016[31]
PFS, OS	AI-based therapy, fulvestrant, and exemestane	ctDNA	383	Turner et al., 2020[32]
PFS	AI-based therapy	cfDNA	103	Zundelevich et al., 2020[33]
PFS, OS	chemotherapy, cyclin-dependent kinase inhibitor and endocrine therapy	ctDNA	59	Muendelin et al., 2021[34]

AI – aromatase inhibitor; cfDNA – cell free DNA; ctDNA – circulating tumor DNA; PFS –progression-free survival; OS – overall survival.

ESR1 gene mutations and progression-free survival rate

The association of plasma *ESR1* mutations in MBC patients with PFS was investigated in 8 studies with 11 outcomes for PFS data. The pooled estimated effect size showed a statistically significant worsened PFS with *ESR1* mutations using the unchanged wild-type (WT) *ESR1* as a comparison group (HR: 1.34; 95% CI: 1.17–1.55; $p < 0.001$, $z = 4.12$) among MBC patients with positive ER, as illustrated in Fig. 2. The heterogeneity level was moderate in the HRs of the individual trials for PFS ($I^2 = 52\%$).

ESR1 gene mutations and overall survival rate

The pooled estimated HR for OS from 6 studies with 7 outcomes using a random effects model was 1.59 (95% CI: 1.31–1.92; $p < 0.001$, $z = 4.71$), suggesting a prognostic link of *ESR1* gene mutations in study participants with ER-positive MBC. The forest plot of the OS analysis is shown in Fig. 3. The heterogeneity level was moderate in the HRs of the individual trials for OS ($I^2 = 71\%$).

Subgroup analysis according to the type of *ESR1* mutations was described in only 3 studies, which reported the association of individual types of mutations with PFS. The estimated pooled HRs with the D538G mutation showed shorter PFS (HR: 1.42; 95% CI: 1.06–2.11; $p = 0.031$) using the WT *ESR1* mutation as a comparator in BC patients assigned to endocrine therapy and the heterogeneity level was low ($I^2 = 2\%$). Unlike Y537S mutation, which showed high heterogeneity ($I^2 = 78\%$) and its association with PFS was statistically nonsignificant (HR: 1.55, 95% CI: 0.61–2.33; $p = 0.354$).

Based on subsequent therapy for MBC patients, the subgroup stratification for AI-based subsequent therapy showed a statistically significant pooled HR of 1.78 (95% CI: 1.37–2.32; $p < 0.001$, $I^2 = 67\%$) for PFS and 1.37 for OS (95% CI: 1.03–1.80; $p = 0.033$, $I^2 = 20\%$). These findings suggest that circulating *ESR1* mutations predict a lower PFS for patients

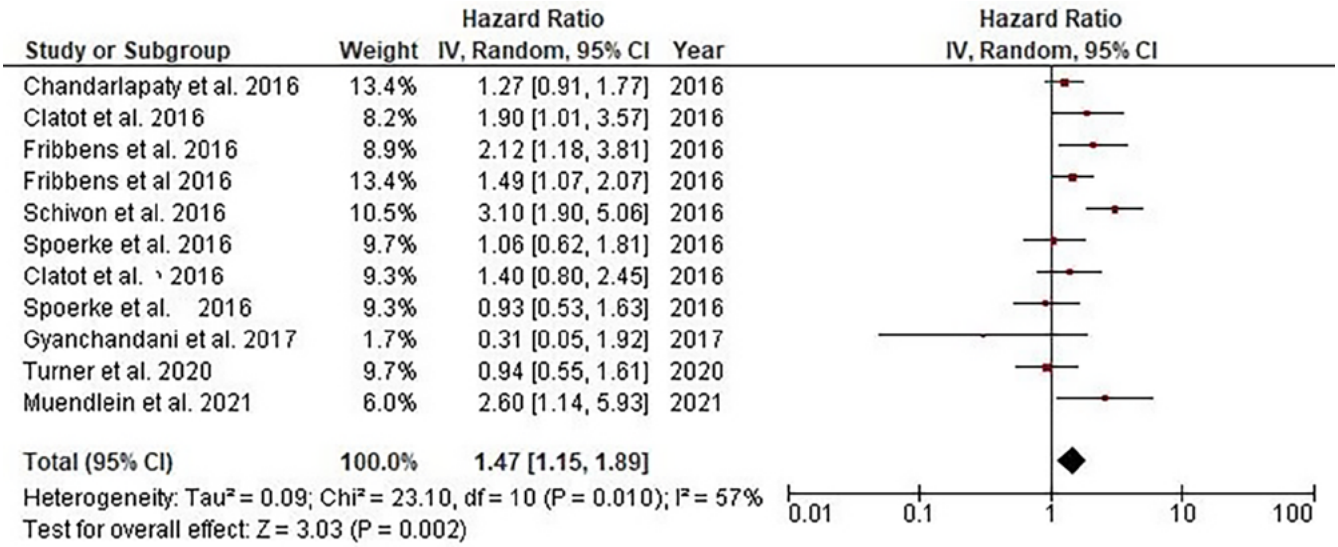

Fig. 2. Forest plot of the *ESR1* gene mutations' effect on the progression-free survival (PFS) rate

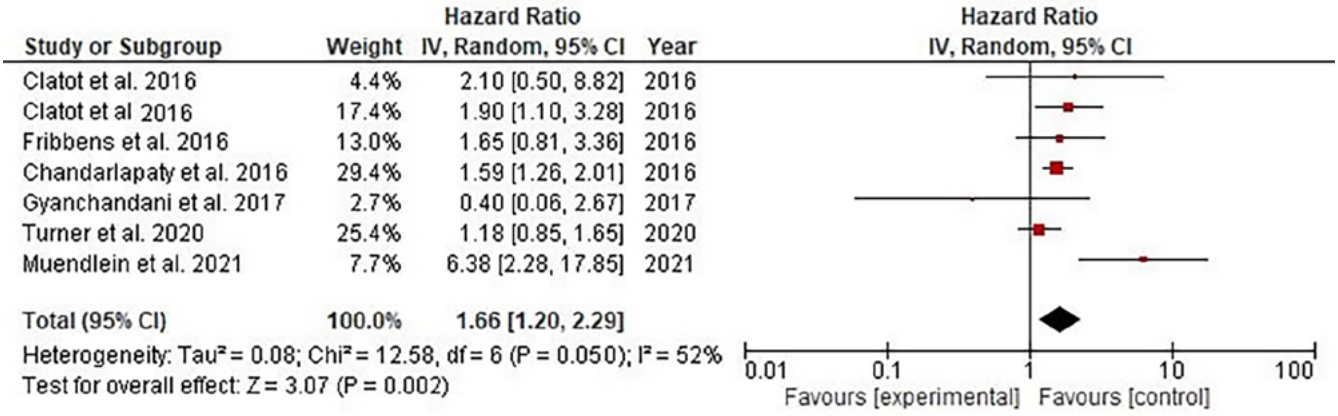

Fig. 3. Forest plot of *ESR1* gene mutations' effect on the overall survival (OS) rate

on subsequent AI therapy. The analysis of fulvestrant subsequent treatment showed a nonsignificant correlation with PFS (HR: 1.56, 95% CI: 0.96–2.16; p = 0.081).

Publication bias

The Egger's regression analysis and Begg's test did not detect any evidence of publication bias among studies investigating the association between *ESR1* mutations (Egger's test: p = 0.592 and 0.741; Begg's test: p = 0.931 and 0.548) and PFS and OS, respectively (Supplementary Table 1). The same was observed during the visual evaluation of Begg's funnel plots, which showed a symmetrical pattern of distribution of the studies (Fig. 4).

Discussion

The present meta-analysis investigated the association between survival status and *ESR1* mutations in plasma among patients with MBC. The pooled HRs for PFS and OS between *ESR1* mutations and WT *ESR1* were 1.48 (95% CI: 1.28–1.73; p < 0.001) and 1.55 (95% CI: 1.30–1.83; p < 0.001), respectively. The results of this study revealed a notable differentiation between BC cases with and without mutations in the *ESR1* gene. Specifically, cases with ESR1 mutations had inferior outcomes in terms of PFS and OS.

Several preclinical trials reported tumor growth suppression with fulvestrant in cell lines with *ESR1* mutations.[35,36] These findings are consistent with our findings of subgroup analysis based on subsequent therapy, where PFS was significantly reduced in patients assigned to subsequent AI therapy with *ESR1* mutations – unlike fulvestrant therapy, which did not significantly favor patients with WT *ESR1*, implying that *ESR1* gene mutations may not be associated with intrinsic or acquired resistance to fulvestrant. Therefore, our findings strengthen the evidence which supports fulvestrant over AI in the treatment of MBC patients.[37,38] Moreover, these outcomes highlight the implication of plasma *ESR1* testing to better guide the selection of endocrine-based treatment.

The advantages of using cfDNA/ctDNA over tissue biopsies for *ESR1* mutation detection have been addressed in several studies. The potential of detecting several additional mutations with the use of cfDNA or ctDNA highlighted the advantage of liquid biopsy.[39,40] In addition,

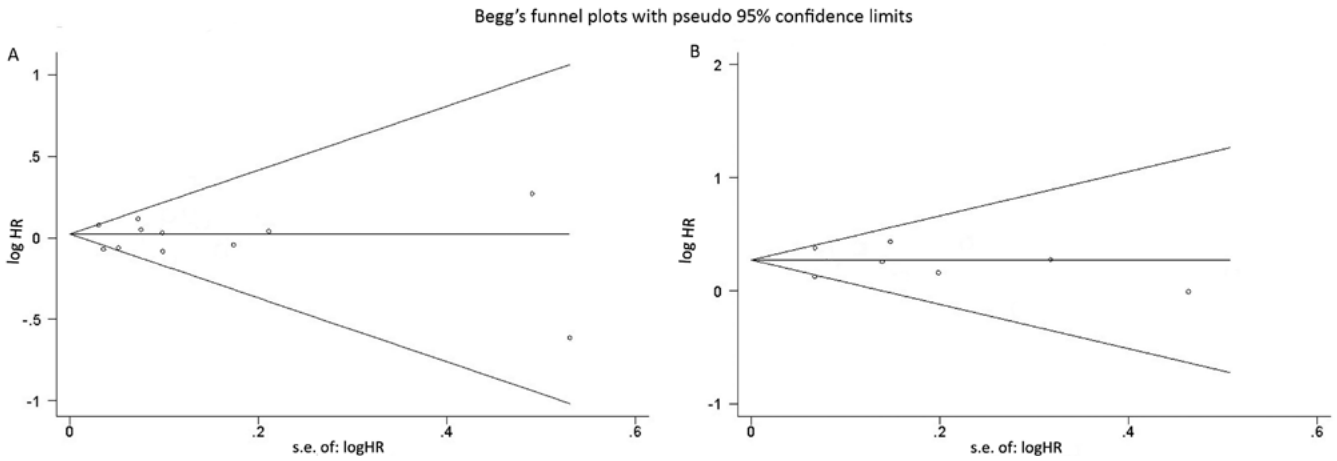

Fig. 4. Begg's funnel plots for publication bias. A. Studies on progression-free survival rate; B. Studies assessing overall survival (OS) rate

the feasibility of serum sample collection enables the sequential assessment of mutations at baseline and during treatment.[39] The increase in *ESR1* mutations during therapy suggests disease progression as a consequence of AI treatment. Upon fulvestrant therapy, if *ESR1* mutated clones decay, this would allow an AI re-challenge in those patients.

We also assessed the effect of *ESR1* mutation types on PFS and OS. The existing analysis showed how distinct *ESR1* mutations (Y537S and D538G) differentially impact patterns of disease progression and patient's survival. Our findings showed that the D538G mutation was associated with shorter PFS compared to WT *ESR1*, but the same was not observed with the Y537S mutation. However, these findings are inconsistent with many basic studies that reported the highest ER activity with Y537S mutations.[41,42] This discrepancy in our findings could be attributed to the limited number of studies included and the high statistical heterogeneity detected (I^2 = 78%). Other mutations, such as E380Q, L536R, Y537N, and Y537C in circulating DNA, have not been investigated in the available literature. Therefore, further studies evaluating all *ESR1* domain mutations and the distinction between them based on their clinical effect are needed.[41,43,44] This meta-analysis highlights the association of *ESR1* mutations with an unfavorable prognosis, as reflected by reduced PFS and OS. The clinical implications of *ESR1* analysis are significant, but issues remain concerning how frequently testing should be performed, taking the patient's age into consideration, and how effectively each type of mutation reacts to treatment. High-quality prospective studies to optimize therapeutic options for regulating ER signaling prior to the onset of extensive disease metastasis would thus be a sensible next step for future trials. Moreover, the literature lacks studies aimed at investigating the potential of *ESR1* mutations as a means of real-time and dynamic monitoring of tumor progression and therapeutic effectiveness in patients with MBC who exhibit resistance to endocrine therapy.

This meta-analysis has several strengths. First, all selected trials for analysis were relevant to our study, which assessed the clinical implications of *ESR1* mutations on disease progression and survival. Thus, the risk of bias is limited. Second, this study provided updated and comprehensive evidence from the literature about an emerging topic in the management of BC and provided guidance for clinicians with patients who have different *ESR1* mutations.

Although our findings are promising, several limitations can also be discussed.

Limitations

The main limitation is the wide diversity between study protocols and the use of potentially biased evidence, with considerable heterogeneity between the trials. Second, the relatively small sample size might affect the certainty of the estimates. Finally, some outcome data were extracted from curves, which might affect the accuracy of the results.

Conclusions

The identification of *ESR1* mutations in circulating DNA analyses has been found to be indicative of a poorer OS in patients who have received prior treatment with an AI. It may seem prudent to consider the analysis of circulating ctDNA for prognosis prediction and directing the choice of endocrine therapy in MBC patients positive for ERs, especially those who did not adequately respond to AI therapy. Moreover, *ESR1* mutations and WT *ESR1* can be considered different subtypes of advanced BC with positive ERs. Future research may help better establish the use of plasma DNA sampling in clinical practice, improve our understanding of the clinical impact of the different types of *ESR1* mutations, and potentially guide therapeutic selection.

Supplementary data

The Supplementary materials are available at: https://doi.org/10.5281/zenodo.10212351. The package includes the following file:

Supplementary Table 1. Publication bias and heterogeneity test results among studies in overall and subgroup analyses.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

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