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Review

The association between type of endocrine therapy and development of estrogen receptor-1 mutation(s) in patients with hormone-sensitive advanced breast cancer: A systematic review and meta-analysis of randomized and non-randomized trials

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ABSTRACT

Background: Breast cancer has, due to its high incidence, the highest mortality of cancer in women. The most common molecular type of breast cancer is the luminal subtype, which expresses estrogen and progesterone receptors and is typically treated with surgery and adjuvant endocrine therapy (ET). Estrogen receptor alpha (ERα), encoded by the estrogen receptor-1 (ESR1) gene, is expressed in approximately 70% of all breast cancers, and ET represents a major treatment modality in ERα-positive cancers. However, resistance to different ET evolves frequently, leading to disease progression or recurrence in ER + breast cancer. Acquired mutations in the Ligand Binding Domain (LBD) of the ERα referred as ESR1 mutations; could be selected by ET itself leading to resistance over the course of ET therapy.

Objective: The goal of this review is to estimate the effect of Aromatase Inhibitors (AIs), Tamoxifen (TAM) and Fulvestrant (FUL) on the development of ESR1 mutations in hormone-sensitive advanced breast cancer.

Methods: A systematic review of qualitative studies published between January 1st, 2007 and March 1st, 2019 was conducted using the PubMed and Thomas Reuters Web of Science databases. Search terms included ESR1 mutations, estrogen receptor, breast cancer, recurrent, metastatic disease, aromatase inhibitors, fulvestrant and tamoxifen. Only full-text studies in English concerning the development of ESR1 mutations and their outcomes on disease progression were included. Selection of studies was performed using predefined data fields, taking study quality indicators into consideration. Inclusion criteria of the study populations were: Ghoncheh et al. (2016) [1] female patients above 18 years; Nielsen et al. (2011) [2] Estrogen-receptor positive (ER +) breast cancer in the advanced setting; Reinert et al. (2017) [3] previous exposure to endocrine therapy including SERDs (preferably Fulvestrant), SERMs (preferably Tamoxifen) or Aromatase Inhibitors.

Results: The current review enrolled 16 articles, including 4 multicentre double blinded RCTs and 12 cohorts and comprising a total of 2632 patients. The overall incidence rate of the ESR1 mutation was 24% (95% CI: 18%–31%). We observed that D538G was the most frequent ESR1 mutation. Several studies showed that prior endocrine therapy (AIs, TAM, FUL) could result in an ESR1 mutation and therapy resistance leading to disease progression or recurrence. Different mechanisms had been implied to explain the underlying ET resistance. One of the key findings of this work is the significant difference in ESR1 mutation incidence between patients with

Abbreviations: AI, Aromatase inhibitor; ctDNA, Circulating tumor DNA; ddPCR, Droplet digital polymerase chain reaction; EDTA, Ethylenediaminetetraacetic acid; ER, Estrogen receptor; ERα, Estrogen receptor alpha; ESR1, Estrogen receptor 1; ET, Endocrine therapy; FUL, Fulvestrant; HR, Hormone Receptor; LBD, Ligand binding domain; NGS, Next generation sequencing; MBC, Metastatic breast cancer; RCT, Randomized controlled trial; SERDs, Selective estrogen receptor down-regulators; SERMs, Selective estrogen receptor modulators; TAM, Tamoxifen

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and without AI therapy (OR: 9.34, 95% CI: 3.28–26.62, $P \leq .001$).
Conclusion: ESR1 mutations are not uncommon phenomenon in patients with hormone-sensitive advanced breast cancer. There is a significant higher incidence rate of ESR1 mutations in patients with previous AI-containing therapeutic regimens, compared to those who received non-AI containing regimes. These ESR1 mutations could lead to the development of complete endocrine resistance to AI, whereas only partial resistance is seen in case of TAM or FUL.

1. Introduction

Breast cancer has the highest mortality in women [1]. The most common subtype is the luminal type, which contributes to 70% of all breast cancers. This molecular subtype expresses the estrogen receptor alpha (ERα), which is encoded by the ESR1 gene. ERα-positivity at diagnosis is a favorable prognostic factor in breast cancer, however breast cancer tends to lose its ERα positivity after 5–6 years [2]. This shift may suggest an important role of the acquired resistance to ET.

Normally, the ERα, which is a nuclear protein, is ligand-dependent. Estrogen binds to the receptor and induces conformational changes, allowing the complex to bind to particular DNA sequences (estrogen response elements). Meanwhile, co-repressors and co-activators could influence the process of gene transcription [3]. Resistance to ET can be divided into two basic patterns, de novo and acquired. ESR1 expression can fluctuate over time; some cells that stain negative at a point of disease evolution, may present a detectable re-expression at another point of time. Nowadays, resistance to ET is an important driver of mortality in breast cancer patients [4]. Recent studies unveiled that these ESR1 mutations lead to constitutive activity of ER, meaning that the receptor is active in absence of its ligand-estrogen, thus conferring partial or complete resistance against ET [5]. A suggested mechanism for this constitutively ligand-independent ERα activity is a shift in helix 12 of the ESR1, resulting in resemblance to the ligand-bound active state of the ER [6]. As a result of this change in ER configuration, co-activators will be able to bind and activate ER in absence of a ligand [7].

There are three major endocrine strategies that constitute the cornerstone of the treatment of ER+ metastatic breast cancer (MCB): Aromatase Inhibitors (AI), Selective Estrogen Receptor Modulators (SERMs), and Selective Estrogen Receptor Downregulators (SERDs). AI depletes systemic estrogen by inhibiting the conversion of androgens to estrogens, thus taking away the stimulating primary ligand of the endocrine receptor. SERMs exhibit their therapeutic action by binding both the intracellular estrogen receptor and the co-repressor proteins. It has a partial ERα agonistic feature (mixed agonistic/antagonistic) with net antagonistic activities on breast tissue. SERDs are a third-class ET that affects the stability of the ER and down regulates the receptor protein. These medications have full antagonistic properties, by binding to the ER and causing receptor destabilization followed by proteosomal degradation [8].

Published studies have not yet been able to determine the exact prevalence of the ESR1 mutations, but set the outer boundaries between 11 and 55% [9]. De novo ESR1 mutations appear to be non-existent or rare in primary tumors [10]. In contrast to primary tumors, a high incidence of mutations has been found in MCB. This suggests that the number of patients with ESR1 mutations will rise if more lines of ET are given [3]. Before the detection of ESR1 mutations in tumor cells, most patients had already a prolonged clinical course; this supports the Darwinian theory, i.e. that ESR1 mutations are selected and enriched during long-term ET, suggesting that these mutations are acquired [3]. According to Takeshita et al. [11] the detection of ESR1 mutations could be used as a potential biomarker predicting the possible effects of ET.

The incidence rate of ESR1 mutations and severity of disease-resistance to ET depends on the type and duration of ET, and potentially other resistance-facilitating mutations. All included studies were considered to be potentially relevant to our review, in which we assessed the incidence rate of ESR1 mutations and their possible outcomes on disease progression. In the current review, we estimated the overall incidence rate of ESR1 mutations in female patients with advanced ER+ breast cancer pretreated with ET. We discussed AIs as a class and TAM and FUL, respectively as an example of SERDs and SERMS. This review was designed to examine the impact of AI(s), TAM and FUL on the development of ESR1 mutations in a large population study with ER+ advanced breast cancer.

2. Material and methods

2.1. Methods of search

The literature search was conducted using two databases: PubMed and Thomson Reuters Web of Science. The following search items were used: [('ESR1 mutation' OR 'ESR mutation' OR 'estrogen receptor mutation') AND (tamoxifen OR fulvestrant OR 'aromatase inhibitors' OR 'endocrine therapy') AND (resistance OR metastasis) AND ('breast cancer')]. A search restriction was determined according to the study date: only articles published between January 1st, 2007 and March 1st, 2019 were included. Only full-text English studies concerning the development of ESR1 mutations and their outcomes on disease progression were included. The selection of studies was performed using predefined data fields, taking study quality indicators into consideration.

2.2. Screening for eligibility criteria

We selected articles with 'ESR1', 'ESR mutation', and/or 'Endocrine Therapy' in the abstract or title through the endnote library search option, after which three reviewers screened the abstracts of the remaining articles independently. The following inclusion criteria were used for this objective: [1] hormone-receptor positive (HR+) breast cancer in metastatic setting; [2] the article should refer to an interventional trial; reviews, lectures and book sections were excluded. [3]; the article should have endocrine therapy including: SERDs (preferably Fulvestrant), SERMs (preferably Tamoxifen) or Aromatase Inhibitors; [4] female patients; [5] only full text English articles were included.

2.3. Study selection

Three reviewers performed the procedure of study inclusion: [1] assessment of each clinical trial was performed independently in an unblended standardized manner; [2] duplicates were removed afterwards; [3] after the independent screening, all the results were compared and the articles with conflict were discussed until agreement was established; [4] the final step in study selection of the remaining articles had been treated separately; the studies that eventually did not meet the inclusion criteria or did not contain useful information for this systematic review had been excluded after consensus. Table 1 provides a detailed overview of the obtained articles, according to the above-mentioned methodological search.

2.4. Data collection process

We developed a data extraction sheet based on the Cochrane Consumers and Communication Review Group's data extraction

Table 1
overview of the included studies with evidence level accordqing to the oxford centre for evidence-based medicine 2011.

	Author	Year	Evidence level
1.	Bartels et al. [16]	2018	III
2.	Chandarlapaty et al. [20]	2016	II
3.	Clatot et al. [26]	2016	III
4.	Fribbens et al. [13] SoFEA-PALOMA3	2016	II
5.	Jeselsohn et al. [22]	2014	III
6.	Lefebvre et al. [18]	2016	III
7.	Merenbakh-lamin et al. [15]	2013	III
8.	Niu et al. [21]	2015	II
9.	Robinson et al. [23]	2013	III
10.	Schiavon et al. [17]	2015	II
11.	Sefrioui et al. [5]	2015	III
12.	Spoerke et al. [14]	2016	II
13.	Takeshita et al. [11]	2015	II
14.	Toy et al. [28]	2013	II
15.	Toy et al. [27]	2017	III
16.	Yanagawa et al. [12]	2017	II

template. Extracted data compromised the type of clinical trial (RCT or non-RCT), characteristics of study-population, number of participants, exclusion of primary disease, immunohistochemical analysis of the tumor, nature of samples (tissue or plasma), methods of mutation analysis (NGS or ddPCR), type and duration of adjuvant ET and outcomes (overall incidence rate of ESR1 mutations, incidence of mutations in relation to ET and most frequent ESR1 mutation).

2.5. Study quality

The Oxford Centre for Evidence-Based Medicine (OCEBM) 2011 v2.1 was used to assess the selected articles for their level of evidence.

2.6. Risk of bias in individual and across studies

Meta-analyses may suffer from several sources of bias. First of all, not all trials lead to a publication, which induces publication bias, and the language of the original publication might give rise to a selection bias. Due to the broad scope of our research questions (incidence of ESR1 in general, and after different hormonal therapy in specific), not only randomized controlled trials, but also case-controlled and even uncontrolled trials were eligible for inclusion in the review. However, reporting bias, confounding and baseline differences might be more pronounced in non-randomized or uncontrolled studies, as compared to randomized controlled trials. For some of the research questions, only a small number of studies could be included in the meta-analysis.

2.7. Methods of data analysis

To estimate the overall incidence of ESR1 mutation, a meta-analysis for proportions was performed estimating the incidence together with a 95% confidence interval. Because of the high diversity in type of studies, patients and therapies, a random effects model was used.

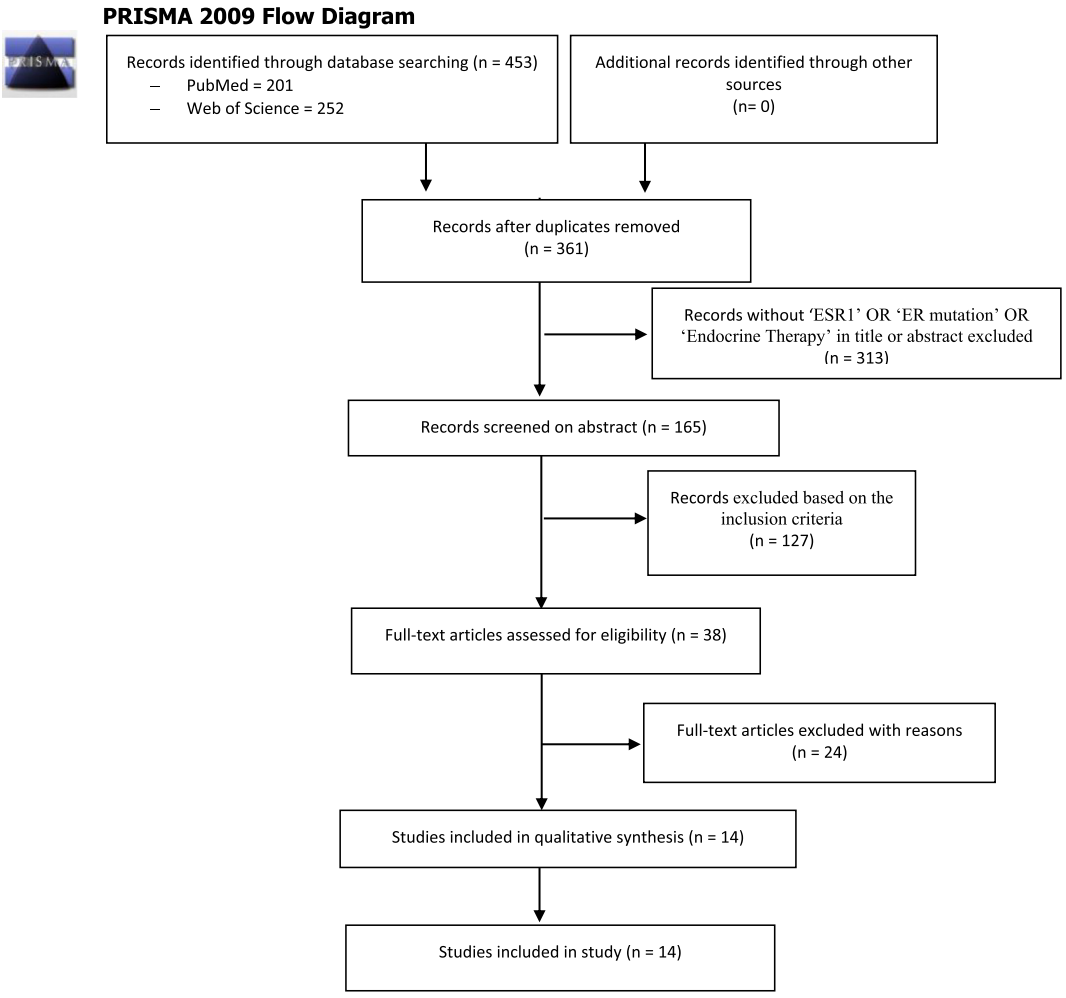

Fig. 1. Prisma flow diagram and the process of data selection.

Heterogeneity is judged by forest plot, Cochran Q and I-squared. Results are presented in a forest plot for proportions.

Incidence of ESR1 mutation is compared between patients with and without AI hormonal therapy with a random effects meta-analysis estimating odds-ratio (OR) and 95% confidence interval. Results are visualized in a forest plot. Only 5 studies report on AI in combination with ESR1, so only these studies were included in this analysis.

3. Results

3.1. Literature search

The literature search with our search criteria found 201 articles in PubMed and 252 articles in Web of Science. A total of 361 articles remained to be examined after the exclusion of the duplicates. Restricting the search to articles with 'ESR1', 'ESR mutation' or 'Endocrine Therapy' in either the title or the abstract left 165 abstracts to be manually screened for inclusion, and 38 articles were read in full. Articles that eventually did not meet the inclusion criteria or did not contain useful information for this systematic review, were discarded after consensus. After this stepwise methodological search, 16 articles were obtained for analysis in this systematic review. Fig. 1 describes the process of data selection using a PRISMA flow chart.

3.2. Study sample characteristics

The studies reviewed included 2913 patients; with 2632 patients eligible for further analysis. A total of 281 patients with primary breast cancer were excluded, including 265 patients from Takeshita et al. [11] and 16 patients from Yanagawa et al. [12]. The selected patients fulfilled the following inclusion criteria: [1] female aged above 18 years; [2] ERα positive breast cancers pre-treated with AI +/− TAM+/− FUL; and [3] advanced breast cancer. A total of 2356 tissue and plasma samples were analysed. Tissue samples were obtained either from local breast recurrence or distant metastasis. Both archived and recent plasma samples were used for ESR1 mutation analysis depending on the type of study analysis.

3.3. Incidence rate of ESR1 mutation

Of the 2632 patients pooled for this analysis, the overall incidence of ESR1 mutation is 24% (95% CI 18%–31%), Fig. 2. However, the included studies demonstrated a considerable variability in the prevalence of these mutations. The most frequent mutations were as followed: D538G, Y537S and Y537N. The exact frequency rates of different ESR1 mutations are shown in Table 2.

Fribbens and colleagues [13] found that the ESR1 mutations were polyclonal in 49.1% of mutant ER (27 of 55) in the SoFEA trial and in 28.6% (26 of 91) in the PALOMA 3 study. This is consistent with the findings of Spoerke et al. [14] who demonstrated polyclonal ESR1 mutations in 40% of the mutant ESR1. The relationship between polyclonal mutations, degree of drug resistance and distant metastasis has not been shown in a large clinical study. Therefore, the clinical significance of polyclonal mutation is not clear yet.

Besides being the most frequent mutation, Merenbakh-Lamin et al. [15] presented in their cell-line study that D538G has the ability to affect the migration of the cancer cells as well, which could imply a more invasive phenotype. These findings of being an invasive phenotype are in line with the study of Bartels et al. [16] that demonstrated a high frequency of D538G mutations in bone metastases (53%). Furthermore, Schiavon et al. [17] demonstrated mutant ESR1 genes in 11% (18/171) of the studied patients; bone metastases were present in 84% of the patients with an ESR1 mutation. In the study of Fribbens et al. [13], prospective-retrospective analysis of ESR1 mutations in archived baseline plasma from the PALOMA3 trial demonstrated a significant association between ESR1 mutations and bone metastases, 87.9% (80/91); (P = .001).

Lefebvre et al. [18] examined the mutational profiles from 216 patients with HR+/HR- MBC. Twelve genes (TP53, PIK3CA, GATA3, ESR1, MAP3K1, CDH1, AKT1, MAP2K4, RB1, PTEN, CBFB, and CDKN2A) were identified as significantly mutated in MBC. This study concluded that the ESR1 mutation was the most frequent mutation in HR+ MBC subgroup (n = 143). In total, 22 mutations were identified in 20 out of 143 patients with HR+/HER2- MBCs (14%) and all patients with mutant ER had received prior endocrine therapy.

The most commonly used method for ESR1 detection is the droplet digital PCR (ddPCR), which can carry out thousands of polymerase chain reactions at the same time [11]. The alternative technique was Next Generation Sequencing (NGS). According to Yanagawa et al. [12] NGS is a better option for detecting rare ESR1 mutations, while ddPCR is often used for the detection of more frequent ESR1 mutations. In the articles under review both techniques were used to determine ESR1 mutation in tissue and plasma samples.

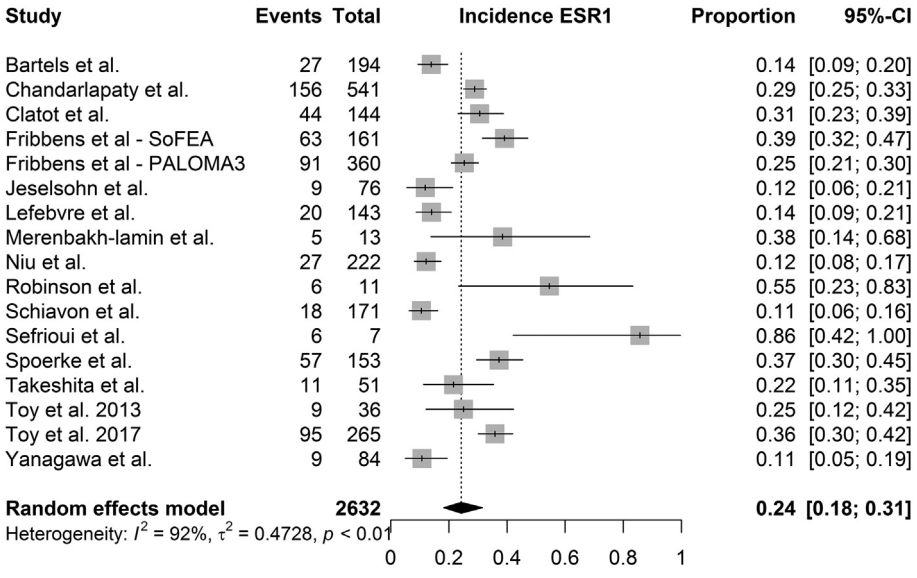

Fig. 2. Forest-plot presentation of overall incidence rate of ESR1 mutation.

Table 2
THE FREQUENCY RATE OF DIFFERENT ESR1 MUTATIONS AND OVERVIEW OF DIFFERENT PARAMETERS IN CORRELATION WITH ESR1 MUTANT.

	Author	Patients with ESR1 mutation n (%)	Type ESR1 mutation	Most frequent	Endocrine therapy	Method
1.	Bartels et al. [16]	27/194 (14%)	D538G, Y537S, Y537N	D538G	AI and/or TAM	NGS
2.	Chandarlapaty et al. [20]	156/541 (29%)	Y537S, D538G	D538G	AI	ddPCR
3.	Clatot et al. [26]	44/144 (30.6%)	D538G, Y537S, Y537N Y537C	D538G	AI	ddPCR
4.	Fribbens et al. [13]					
	SoFEA-	63/161 (39%)	D538G, Y537N,	D538G	AI	ddPCR
	PALOMA3	91/360 (25%)	Y537S	D538G	TAM and/or AI	ddPCR
5.	Jeselsohn et al. [22]	9/76 (12%)	D538G, Y537S, Y537N	Y537N	AI and/or TAM and/or FUL	NGS
6.	Lefebvre et al. [18]	20/143 (14%)	NA	NA	NA	NGS
7.	Merenbakh-lamin et al. [15]	5/13 (38%)	D538G	NA	AI, TAM, FUL	NGS
8.	Niu et al. [21]	27/222 (12%)	Y537, D538	Codon Y537	At least one line AI	NGS
9.	Robinson et al. [23]	6/11 (55%)	L536Q, Y537S, D538G	NA	AI, TAM, FUL	NGS
10.	Schiavon et al. [17]	18/171 (11%)	Y537S, Y537C, Y537N	D538G	AI and/or TAM	ddPCR
11.	Sefrioui et al. [5]	6/7 (86%)	NA	NA	AI, TAM	ddPCR, NGS
12.	Spoerke et al. [14]	57/153 (37%)	D538G, Y537S, E380Q	D538G	AI	ddPCR
13.	Takeshita et al. [11]	11/51 (22%)	Y537S, D538G, Y537N	Y537N	AI, TAM	ddPCR
14.	Toy et al. [28]	9/36 (25%)	Y537S, D538G, Y537N	NA	AI, TAM, FUL	NGS
15.	Toy et al. [27]	95/265 (36%)	D538G, Y537S, E380Q	D538G	NA	NGS
16.	Yanagawa et al. [12]	9/84 (11%)	Y537S, Y537N, D538G	D358G	AI, AI and TAM/FUL	NGS

3.4. Impact of SERM, AI and SERD on ESR1 mutations

Association between the type of endocrine therapy and the development of ESR1 mutations is nowadays a hot topic. Recent studies reported that the occurrence of ESR1 mutations are frequently found in HR+ recurrent breast cancers pretreated with endocrine therapy. On the other hand, these mutations are rare in primary breast cancers. Regrowth or recurrence of breast cancer after a good initial response to endocrine therapy suggests an acquired resistance to ET, which is often caused by an ESR1 mutation [19].

Only in 7 out of 14 reviewed studies, the type of previous endocrine therapy was clearly indicated. In these studies, 1274 patients had received prior AI-containing therapy of whom 407 developed an ESR1 mutation, while only 4 ESR1 mutations were seen in 140 patients pretreated with endocrine therapy other than AI. Analysis of these data showed a clear association between the development of an ESR1 mutation and prior estrogen deprivation therapy by aromatase inhibitors. As illustrated in Fig. 3, the incidence of ESR1 mutation is higher in patients with an AI-containing therapy (OR 9.34 95%CI: 3.26–26.62).

In the PALOMA3 trial, ESR1 mutations were found in the plasma of 25.3% of the patients (91 of 360). These 91 patients with an ESR1 mutation had different treatment regimens (AI monotherapy, AI + TAM, TAM monotherapy). 41 out of 91 patients with an ESR1 mutation were treated with AI only, while 49 patients had received combination therapy of AI and tamoxifen [13]. The clinical impact of patient's race, age, site of metastasis or susceptibility to previous ET is minimal regarding the development of ESR1 mutations. Only the ECOG performance status of daily activities accounts for a small variation in frequency of ESR1 mutations.

Clinical studies showed a relationship between the line of AI therapy and the acquirement of ESR1 mutation i.e. ESR1 mutations are rarely the result of exposure to AI in the adjuvant setting [17,20]. Since the amount of tumor mass in this micrometastatic cancer disease is very low, mutant subclones in early-stage cancer disease cannot be selected by ET. ESR1 mutations are therefore very rare in micrometastatic tumors. In contrast, Chandarlapaty et al. [20] discovered a threefold increase in the prevalence of ESR1 mutations in patients with AI therapy for macro-metastatic disease. These findings are consistent with results from Schiavon et al. [17].The difference between AI-exposure in metastatic and early stage cancer disease settings was significant in both studies individually, but due to the large heterogeneity between the two studies, the overall effect is not significant in a meta-analysis (OR 15.5 95%CI: 0.67–360, $P = .087$). Data of the corresponding studies are illustrated in Fig. 4.

In the reviewed articles, ESR1 mutations were demonstrated in very low frequency in patients who received prior therapy of tamoxifen monotherapy: Niu et al. [21] 3.7% (1/27); the PALOMA 3 trial [13] 1.1% (1/91); Jeselsohn et al. [22] 22% (2/9); Robinson et al. [23] 0% (0/6). In Schiavon et al. [17], no ESR1 mutations were observed in 22 patients who had only received tamoxifen monotherapy (0/22, 95% CI 0–15%), while 18 patients in whom ESR1 mutations were observed had received prior AI treatment. Large clinical studies are needed to examine the development of ESR1 mutation in context of tamoxifen monotherapy.

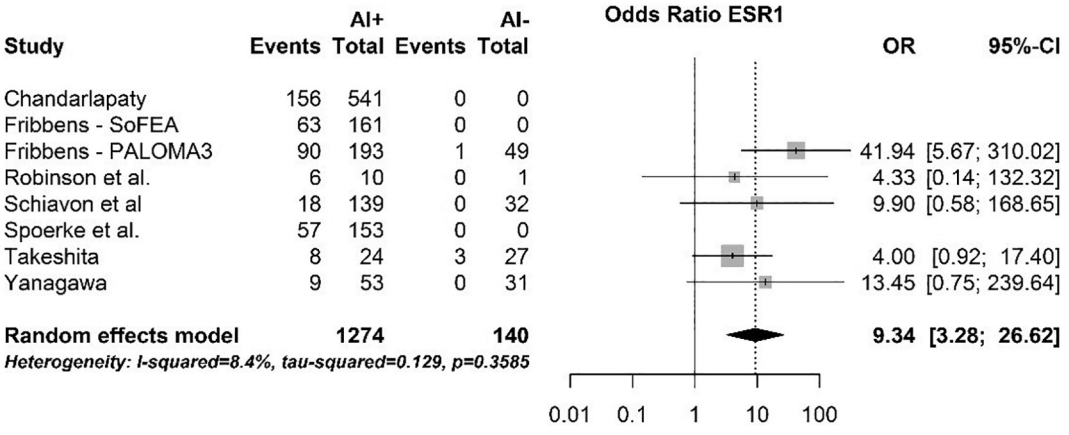

Fig. 3. Forest-plot presentation of the correlation between AI versus non-AI containing treatment and incidence of ESR1 mutation.

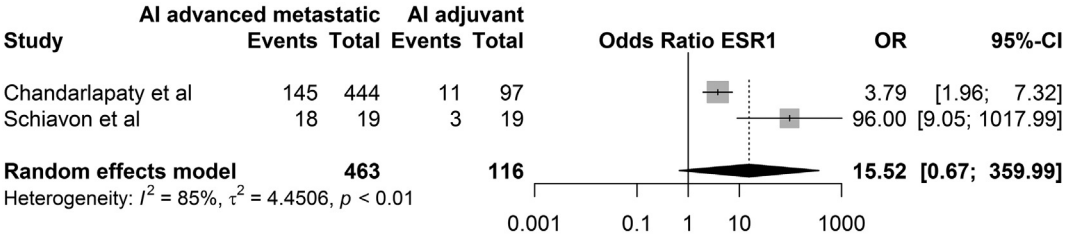

Fig. 4. Exposure to AI in different tumor settings.

In our reviewed articles, there are no data available of fulvestrant monotherapy causing ESR1 mutations, therefore it is impossible to make a statement about the direct effect of fulvestrant on the occurrence of ESR1 mutations. Robinson et al. [23] suggest that it is unlikely that ESR1 mutations arise owing to anti-estrogen therapies (FUL, TAM), as the mutated variants are still sensitive to these therapies. Spoerke et al. [14] showed that there was no coherent pattern of increase in ESR1 mutation allele frequency during fulvestrant treatment in patients with progressive disease.

Exposure time to endocrine therapy and absolute time are important cofactors in the development of acquired ER mutation in HR+ breast cancer. In the PALOMA3 trial [24], the mean time from primary diagnosis to the first relapse in study and control groups was five years (range = 2–10 years). On the other hand, the EROS 1 trial demonstrated that 19% (4/21) of the patients with recurrent breast cancer who developed acquired resistance after 5–10 years, possessed a mutant ER [25]. Clatot et al. [26] showed that the median time of AI exposure was significantly longer in patients with an ESR1 mutation than in patients without an ESR1 mutation (15 vs 10.5 months, respectively, P = .02). Until now, no study has examined the time needed for the development of ESR1 mutation after exposure to endocrine therapy. The time factor should have a significant impact on clinical outcomes, especially in young aged patients.

3.5. ESR1 mutation as therapeutic target

Resistance to estrogen deprivation by aromatase inhibitors is a possible consequence of ESR1 mutations, since these mutations allow breast tumors to proliferate independently of the ligand estrogen due to the constitutive activity of the ER. Several preclinical studies showed a relative resistance of the ESR-LBD mutations to FUL and TAM. However, inhibition of these mutants with higher doses of FUL or TAM or more-potent SERDs or SERMs might benefit patients with LBD-mutated ERα breast cancer [13,15,27,28]. In the retrospective analysis of Fribbens et al. [13], ESR1 mutations in archived baseline plasma from the SoFEA trial and PALOMA3 trial were investigated. The SoFEA trial compared Exemestane with FUL containing treatment regimes in patients with prior AI hormonal therapy. Patients with a mutant ESR1 had a relatively improved PFS with FUL compared to Exemestane, which implies a relative resistance to Exemestane and a relative sensitivity to Fulvestrant. In contrast, patients without detected ESR1 mutations may derive further benefit from both Exemestane and Fulvestrant. In general, this study suggests that breast cancers with ESR1 mutations show selective sensitivity to FUL, but overall with a moderately worse PFS compared to wild-type cancers. The PALOMA3 trial [24] compared FUL and placebo with FUL and Palbociclib in women with HR+, HER2-MCB whose disease had progressed after previous endocrine therapy including AI, TAM or a combination of AI and TAM. This trial concluded that FUL plus Palbociclib gives a significant improvement in PFS outcomes compared to FUL plus placebo, regardless of endocrine resistance and mutational status. The finding that the beneficial effects might be independent of hormone-receptor expression level strongly suggests that the mechanism of action of CDK4/CDK6 inhibitors could be unrelated to established ER-associated resistance pathways. Toy et al. [27] compared the effects of SERDs on partial resistance conferred by mutant ER. FUL appears to be very effective against mutant ER in vitro, but less potent when compared to mutant ER in vivo. However, FUL is able to fully inhibit wild-type, E380Q and S463P ESR-driven breast cancers while Y537S mutants were not fully inhibited by FUL, despite higher doses. On the other hand, oral SERDS, AZD9496 and GDC-0810, were able to fully block growth of a mutant ER including those driven by Y573S. This could be explained by the poor pharmacokinetic properties of FUL and the incomplete receptor occupancy in vivo. However, analysis of patient-derived xenograft demonstrated partial tumor-growth inhibition with AZD9496. This may indicate that not all mutant tumors were exclusively dependent on ER signaling for their growth.

According to Jeselsohn et al. [22] ESR1 mutations were not associated with lower PFS after FUL treatment in comparison to those with wild-type ESR1. This may suggest that these mutations are not strongly associated with resistance to FUL treatment. On other hand, Clatot et al. [26] demonstrated a clear association between ESR1 mutation and AI resistance and they observed a poor outcome for both PFS and OS. The median OS and PFS were lower in patients with a circulating ESR1 mutation compared to patients without a mutation.

4. Discussion

Breast tumors are characteristically heterogeneous at cellular, molecular, and genetic/epigenetic levels. In general, patients with hormone-sensitive disease show an initial benefit from ET; but resistance can develop due to the selective pressure of ET [17,29]. However, the potential for both spatial and temporal heterogeneity in ER expression is still not well understood.

The included studies used different hormonal therapeutic strategies. Most patients were pre-treated with at least a member of AIs, possibly in addition with other hormonal agents such as TAM and/or FUL. This heterogeneity of treatment regimens leads to inconclusive results concerning the effects of the different treatment modalities on the emergence of ESR1 mutations: are combinations of hormonal agents responsible for these ESR1 mutations or are these mutations caused by one single agent? Table 2 provides an overview of different parameters in correlation to ESR1 mutations, including hormonal therapies used in the different studies.

Resistance develops in 25% of the patients with primary breast cancer and approximately in all the patients with MBC [30]; one of the causes of this resistance are mutations in the ligand-binding domain of the ERα (LBD ESR1-mutations, sometimes referred as 'hotspot mutations'). In this review, we refer to this gain-of-function mutation as ESR1 mutation. These mutations are known to promote tumor growth, induce resistance to ET and possibly enhance the metastatic (anchorage-independent) capacity of the tumor [30]. The three most frequent mutations in the LBD are clustered in the codons 537 and 538: Y537S, Y537N and D538G. Significant difference between these mutations regarding resistance and prognosis has been observed, e.g. Y537S is more resistant than D538G. In the BOLERO trial: patients with the Y537S have a shorter overall survival than patients with the D538G mutation [3,31]. On the other hand, the most frequent mutation, D538G, has a

more invasive profile which leads to an enhanced migratory ability of the tumor cells [15]. Besides the mutations in the LBD of the ERα, there are many other molecular alterations of the ERα, such as amplifications, deletions, splice variant etc. We focused only on the mutations in the LBD of the ER. We reported an overall prevalence rate of ESR1 mutation of 24% (95% CT 18%–31%) of the study samples. Our data analysis showed that D358G, Y537S and Y537N were the most frequent mutations, consistent with results from other studies.

4.1. Plausible mechanisms of resistance with TAM, AIs and FUL

The resistance to TAM and FUL depends on changes in the interaction between hormonal agents and the ERα, while resistance to AI is caused by the estrogen-independent activity of the ERα. Considering that the affinity of the ERα varies for different ligands, it is possible that all LBD mutations will have a different effect on each particular receptor-ligand interaction [8].

4.2. Tamoxifen

TAM is a Selective Estrogen Receptor Modulator (SERM). It has a selective tissue-specific antagonistic effect on ERα of the mammary tissue while exhibiting agonistic pro-estrogenic properties on the uterine tissue [32]. This explains the seemingly paradoxical elevated incidence in endometrial carcinoma during TAM therapy [33]. TAM inhibits the binding of co-activators to the ERα with subsequent inactivation of the ER [15]. There is a considerable amount of controversy and uncertainty about the mechanisms leading to TAM resistance. TAM resistance is an important challenge in the current treatment of breast cancer. Almost one in every three women with primary disease treated with adjuvant tamoxifen will acquire resistance to therapy over a variable period of time [32]. Resistance to TAM could be caused by genomic alterations other than mutations(s) to ESR1 such as miRNA [34] and amplifications of ESR1 [2], but this goes beyond the scope of this review. Jeselsohn et al. [7] [22] stated that resistance to TAM is not a complete resistance like in case of AIs, but rather a partial resistance. Therefore, increasing therapeutic dosage might be sufficient to overcome the resistance.

4.2.1. We will discuss two specific resistance mechanisms

First, ESR1 mutations cause a shift of helix 12 (H12) from the ER which results in the stabilization of the receptor in the agonist 'activated' mode. The conformational change of the receptor will increase the level of binding-affinity to the co-activators. Therefore, the co-activators could bind the receptor despite the absence of the ligand [7]. On the other hand, the binding-affinity for TAM will be diminished; subsequently decreasing the potency of TAM therapy. In addition, D538G mutation causes a subtler active state of the ER in comparison with Y537S; hence leading to a stronger resistance if the Y537S mutation is present [7,15,22].

Secondly, certain ESR1 mutations may enhance the cross-talking between the ER and other growth factor receptors, especially with IGF1R which is responsible for the insulin growth factor signalization pathway. This interaction between the receptors will alter the anti-proliferative effects of TAM by influencing the transcriptional activity of the ER. The sensitivity of ER to TAM will be restored if the IGF1R activity has been blocked. However, not all mutations could exert their effect via the above-mentioned mechanism. This type of receptor resistance has been proven for Y537S and D538G mutations. The Y537S mutation has been shown so far to be the most resistant clone [35].

4.3. Aromatase inhibitors

Aromatase is an enzyme of the cytochrome P450 family that is encoded by the CYP19 gene. It is expressed in tissues such as the liver, muscles, brain, subcutaneous fat and breast tissue. This enzyme converts the adrenal substrate androstenedione to estrogen in peripheral tissues of the body. AIs reduce the circulating estrogen by inhibition of estrogen synthesis in peripheral tissue by 90% or more but without effect on estrogen production in the ovaries. The rationale for the use of these compounds in postmenopausal patients is that estrogen in this set of patients is produced by aromatase in peripheral tissues and in the tumor [36]. AIs can induce ER resistance by mechanisms other than those caused by TAM or FUL. This could explain why breast cancer patients, who have developed resistance to AIs, respond to other anti-estrogen therapies.

Robinson et al. [23] studied 11 patients with breast cancer of which 6 patients harbored a hotspot ESR1 mutation. The six patients were all treated with estrogen deprivation (AI or oophorectomy) in combination with anti-estrogen therapies (TAM, FUL). Estrogen response element (ERE) reporter levels were evaluated after a 24 h exposure of the steroid hormone-deprived cells to β estradiol. The LBD-localized ESR1 mutations showed a strong activation of ERE, which was not significantly augmented with β estradiol. Consequently, they showed that these LBD-ESR1 mutations result in constitutive estrogen-independent ERα activity. Their conclusion supports many other (pre)-clinical studies, regarding the theory of ligand-independent activity of the mutated ER. Wild-type estrogen receptors are ligand-dependent for activation and receptor transcription. In conclusion, estrogen deprivation could ensure inactivity of wild-type ER, with little or no effect on mutated ER.

Taken together, one can state that the LBD-ESR1 mutations develop predominantly in the context of estrogen deprivation, i.e. therapy with AI or oophorectomy, which may promote tumor growth [30,37]. Our analysis showed a significant difference in ESR1 mutation incidence between patients with and without AI (OR: 9.34 (95% CT: 3.28–26.62, $P \leq .001$), as shown in Fig. 3.

4.4. Fulvestrant

FUL is a Selective Estrogen Receptor Degrader (SERD). It has the capacity to bind, block and degrade the estrogen receptor. These actions cause complete inhibition of estrogen signaling through the estrogen receptor. FUL is capable of degrading the ERα and stopping the up-regulation of proliferation when the ERα becomes constitutively active [38]. As well as for TAM, there is a considerable amount of controversy and uncertainties about the resistance mechanism of FUL. One of the main mechanisms of resistance to FUL is due to a shift of helix 12 from the ER and subsequently a partial resistance of the mutated receptor to FUL [22]. As for TAM, it is important to keep in mind that not every resistance to FUL therapy is induced by ESR1 mutations [17]. In contrast, Spoerke et al. [14] showed that ESR1 mutations were not associated with lower PFS after FUL treatment in comparison to those with wild-type ESR1. This may suggest that these mutations are not strongly associated with resistance to FUL treatment.

4.5. Strength, clinical implications and limitations of the current review

4.5.1. Strength

All included studies were considered to be potentially relevant to our review that assessed the incidence rate of ESR1 mutations and their outcomes on disease progression. This provides a rather (good) homologue quality of the studies included in this review, which limits the risk of bias. Present literature review discusses an emerging topic in breast cancer treatment and can be a basis for further research. According to our knowledge, this is the largest review that carries out a comparative analysis between hormonal therapies and ESR1 mutation.

4.6. Clinical implications

As mentioned above, ESR1 mutations are subjected to selection pressure from endocrine therapy. Due to this selection, the disease may relapse after a few years or there will be a disease progression during

endocrine treatment. ESR1 mutations are not the only reason for endocrine resistance. Schiavon et al. [17] stated that 93% of the patients included in the study had at least one mutation other than ESR1 mutation. Thus, it is of great importance not to attribute all hormone-resistant breast cancers to ESR1 mutation. In future, analysis of ESR1 mutation should be considered in hormone-sensitive breast cancer to avoid disease progression and unnecessary endocrine therapy. In patients with positive ESR1 mutation, it is recommended to adapt the endocrine therapy into a FUL containing regimen; if needed, it is possible to enhance the normally prescribed dose within the therapeutic range.

4.7. Limitations

The main limitation of this meta-analysis, as with any review, is a lot of diversity in therapeutic strategies. There seems to be neither a standard duration nor a standard endocrine therapy strategy for the treatment of hormone positive breast cancer in the metastatic setting. Because of this heterogeneity, it was difficult to correlate the development of ESR1 mutations with a specific hormonal strategy. The different studies show a large variability regarding the prevalence in percentage of ESR1 mutations (6% to 55%). The wide range in incidence rate of ESR1 mutation could be attributed to heterogeneity in the study populations.

According to the hypothesis of this review, there was a risk for selection bias because the selected patients had advanced breast cancer. Other sources of bias in the selection criteria cannot be ruled out. One is, of course, the publication bias for positive findings. A well-designed study that ends up showing nothing, even if it conclusively shows no difference, tends not to get published. For some of the research questions, only a small number of studies could be included in the meta-analysis. The quality of the studies varied. Due to the broad scope of our research questions (incidence of ESR1 in general, and after different hormonal therapy in specific), not only randomized controlled trials, but also case-controlled and even uncontrolled trials were eligible for inclusion in the review.

5. Conclusion

In this review, we analysed 16 randomized and non-randomized clinical trials published between January 1st, 2007 and March 1st, 2019. Present meta-analysis showed that the overall incidence rate of ESR1 mutations in advanced ER + breast cancers was 24%, with D538G as the most frequently found ESR1 mutation. A complete resistance could be developed in AI-containing regimes, whereas partial resistance will occur in case of TAM or FUL. There is a significant difference in ESR1 incidence between patients with and without AI-containing treatment. The clinical implications of ESR1 analysis are significant, while questions remain as to the optimal frequency of testing, taking the age of the patient into account, and mutation-specific therapeutic susceptibilities. A logical next step for future trials would thus be high-quality prospective studies to optimize the therapeutic strategies in controlling ER signaling before the occurrence of widespread disease metastasis.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

[1] M. Ghoncheh, Z. Pournamdar, H. Salehiniya, Incidence and mortality and epidemiology of breast Cancer in the world, Asian Pacific J. Cancer Prevention 17 (S3) (2016) 43–46.
[2] K.V. Nielsen, B. Ejlertsen, S. Muller, S. Moller, B.B. Rasmussen, E. Balslev, et al., Amplification of ESR1 may predict resistance to adjuvant tamoxifen in postmenopausal patients with hormone receptor positive breast cancer, Breast Cancer Res. Treat. 127 (2) (2011) 345–355.
[3] T. Reinert, E.D. Saad, C.H. Barrios, J. Bines, Clinical implications of ESR1 mutations in hormone Receptor Positive advanced breast, Front. Oncol. 7 (2017).
[4] R. Clarke, J.J. Tyson, J.M. Dixon, Endocrine resistance in breast cancer - An overview and update, Mol. Cell. Endocrinol. 418 (2015) 220–234.
[5] D. Sefrioui, A. Perdrix, N. Sarafan-Vasseur, C. Dolfus, A. Dujon, J.M. Picquenot, et al., Short report: monitoring ESR1 mutations by circulating tumor DNA in aromatase inhibitor resistant metastatic breast cancer, Int. J. Cancer 137 (10) (2015) 2513–2519.
[6] D.M. Tanenbaum, Y. Wang, S.P. Williams, P.B. Sigler, Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains, Proc. Natl. Acad. Sci. U. S. A. 95 (11) (1998) 5998–6003.
[7] R. Jeselsohn, C. De Angelis, M. Brown, R. Schiff, The evolving role of the estrogen receptor mutations in endocrine therapy-resistant breast Cancer, Curr. Oncol. Rep. 19 (5) (2017).
[8] B. Button, B. Park, ESR1 mutations: Piece de resistance, Genes & Diseases. 3 (2) (2016) 124–129.
[9] S. Tabarestani, M. Motallebi, M.E. Akbari, Are estrogen receptor genomic aberrations predictive of hormone therapy response in breast Cancer? Iranian J. Cancer Preven. 9 (4) (2016).
[10] The Cancer Genome Atlas N, D.C. Koboldt, R.S. Fulton, M. MD, H. Schmidt, J. Kalicki-Veizer, et al., Comprehensive molecular portraits of human breast tumours. Nature, 490 (2012), p. 61.
[11] T. Takeshita, Y. Yamamoto, M. Yamamoto-Ibusuki, M. Tomiguchi, A. Sueta, K. Murakami, et al., Analysis of ESR1 and PIK3CA mutations in plasma cell-free DNA from ER-positive breast cancer patients, Oncotarget. 8 (32) (2017) 52142–52155.
[12] T. Yanagawa, N. Kagara, T. Miyake, T. Tanei, Y. Naoi, M. Shimoda, et al., Detection of ESR1 mutations in plasma and tumors from metastatic breast cancer patients using next-generation sequencing, Breast Cancer Res. Treat. 163 (2) (2017) 231–240.
[13] C. Fribbens, B. O'Leary, L. Kilburn, S. Hrebien, I. Garcia-Murillas, M. Beaney, et al., Plasma ESR1 mutations and the treatment of estrogen receptor-positive advanced breast Cancer, J. Clin. Oncol. 34 (25) (2016) 2961–2968.
[14] J.M. Spoerke, S. Gendreau, K. Walter, J.H. Qiu, T.R. Wilson, H. Savage, et al., Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant, Nat. Commun. 7 (2016).
[15] K. Merenbakh-Lamin, N. Ben-Baruch, A. Yeheskel, A. Dvir, L. Soussan-Gutman, R. Jeselsohn, et al., D538G mutation in estrogen receptor-alpha: a novel mechanism for acquired endocrine resistance in breast Cancer, Cancer Res. 73 (23) (2013) 6856–6864.
[16] S. Bartels, M. Christgen, A. Luft, S. Persing, K. Jodecke, U. Lehmann, et al., Estrogen receptor (ESR1) mutation in bone metastases from breast cancer, Mod. Pathol. 31 (2018) 56–61.
[17] G. Schiavon, S. Hrebien, I. Garcia-Murillas, R.J. Cutts, A. Pearson, N. Tarazona, et al., Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer, Sci. Transl. Med. 7 (313) (2015).
[18] C. Lefebvre, T. Bachelot, T. Filleron, M. Pedrero, M. Campone, J.C. Soria, et al., Mutational profile of metastatic breast cancers: a retrospective analysis, PLoS Med. 13 (12) (2016) e1002201.
[19] L. Angus, N. Beije, A. Jager, J.W.M. Martens, S. Sleijfer, ESR1 mutations: moving towards guiding treatment decision-making in metastatic breast cancer patients, Cancer Treat. Rev. 52 (2017) 33–40.
[20] S. Chandarlapaty, D. Chen, W. He, P. Sung, A. Samoila, D.Q. You, et al., Prevalence of ESR1 mutations in cell-free DNA and outcomes in metastatic breast Cancer a secondary analysis of the BOLERO-2 clinical trial, Jama Oncology. 2 (10) (2016) 1310–1315.

[21] J.X. Niu, G. Andres, K. Kramer, M.N. Kundranda, R.H. Alvarez, E. Klimant, et al., Incidence and clinical significance of ESR1 mutations in heavily pretreated metastatic breast cancer patients, Oncotargets and Therapy. 8 (2015) 3323–3328.
[22] R. Jeselsohn, R. Yelensky, G. Buchwalter, G. Frampton, F. Meric-Bernstam, A.M. Gonzalez-Angulo, et al., Emergence of constitutively active estrogen receptor-alpha mutations in pretreated advanced estrogen receptor-positive breast cancer, Clin. Cancer Res. 20 (7) (2014) 1757–1767.
[23] D.R. Robinson, Y.M. Wu, P. Vats, F.Y. Su, R.J. Lonigro, X.H. Cao, et al., Activating ESR1 mutations in hormone-resistant metastatic breast cancer, Nat. Genet. 45 (12) (2013) 1446–U197.
[24] M. Cristofanilli, N.C. Turner, I. Bondarenko, J. Ro, S.A. Im, N. Masuda, et al., Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial, Lancet Oncol. 17 (4) (2016) 425–439.
[25] O. Najim, M. Huizing, K. Papadimitriou, X.B. Trinh, P. Pauwels, S. Goethals, et al., The prevalence of estrogen receptor-1 mutation in advanced breast cancer: the estrogen receptor one study (EROS1), Cancer Treatment and Research Communications. 19 (2019) 100123.
[26] F. Clatot, A. Perdrix, L. Augusto, L. Beaussire, J. Delacour, C. Calbrix, et al., Kinetics, prognostic and predictive values of ESR1 circulating mutations in metastatic breast cancer patients progressing on aromatase inhibitor, Oncotarget. 7 (46) (2016) 74448–74459.
[27] W. Toy, H. Weir, P. Razavi, M. Lawson, A.U. Goeppert, A.M. Mazzola, et al., Activating ESR1 mutations differentially affect the efficacy of ER antagonists, Cancer Discovery. 7 (3) (2017) 277–287.
[28] W. Toy, Y. Shen, H. Won, B. Green, R.A. Sakr, M. Will, et al., ESR1 ligand-binding domain mutations in hormone-resistant breast cancer, Nat. Genet. 45 (12) (2013) 1439–U189.
[29] C.A. Miller, Y. Gindin, C. Lu, O.L. Griffith, M. Griffith, D. Shen, et al., Aromatase inhibition remodels the clonal architecture of estrogen-receptor-positive breast cancers, Nat. Commun. 7 (2016).
[30] R. Jeselsohn, G. Buchwalter, C. De Angelis, M. Brown, R. Schiff, ESR1 mutations-a mechanism for acquired endocrine resistance in breast cancer, Nat. Rev. Clin. Oncol. 12 (10) (2015) 573–583.
[31] A. Bahreini, Z. Li, P. Wang, K.M. Levine, N. Tasdemir, L. Cao, et al., Mutation site and context dependent effects of ESR1 mutation in genome-edited breast cancer cell models, Breast Cancer Res. 19 (1) (2017) 60.
[32] P.G. Alluri, C. Speers, A.M. Chinnaiyan, Estrogen receptor mutations and their role in breast cancer progression, Breast Cancer Res. 16 (6) (2014).
[33] A. Fugh-Berman, S. Epstein, Tamoxifen: disease prevention or disease substitution? Lancet. 340 (8828) (1992) 1143–1145.
[34] T. Joshi, D. Elias, J. Stenvang, C.L. Alves, F. Teng, M.B. Lyng, et al., Integrative analysis of miRNA and gene expression reveals regulatory networks in tamoxifen-resistant breast cancer, Oncotarget. 7 (35) (2016) 57239–57253.
[35] L. Gelsomino, G.W. Gu, Y. Rechoum, A.R. Beyer, S.M. Pejerrey, A. Tsimelzon, et al., ESR1 mutations affect anti-proliferative responses to tamoxifen through enhanced cross-talk with IGF signaling, Breast Cancer Res. Treat. 157 (2) (2016) 253–265.
[36] K. Altundag, N.K. Ibrahim, Aromatase inhibitors in breast cancer: an overview, Oncologist 11 (6) (2006) 553–562.
[37] S. Oesterreich, N.E. Davidson, The search for ESR1 mutations in breast cancer, Nat. Genet. 45 (12) (2013) 1415–1416.
[38] M.R. Nathan, P. Schmid, A review of Fulvestrant in breast Cancer, Oncol Ther. 5 (1) (2017) 17–29.