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Review

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Endocrine therapy in metastatic breast cancer-more than just CDK4/6 inhibitors

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Abstract

Advanced hormone receptor-positive breast cancer is one of the women's most common malignant diseases and remains incurable despite recent therapeutic innovations. The dependence of hormone receptor-positive breast cancer on hormonal growth signals offers the possibility of inhibiting this signaling pathway using anti-hormonal therapy. Nevertheless, the development of resistance to antitumoral drugs remains a challenge. Molecularly-targeted substances significantly improve survival rates and (as in the case of cyclin-dependent kinase 4 and 6 inhibitors) are widely used in clinical practice and enhance endocrine therapy's efficacy. Agents such as everolimus, alpelisib, and capivasertib target the phosphoinositide 3 kinase/protein kinase B/mammalian target of rapamycin pathway, which is a promising approach to overcoming endocrine resistance. Novel therapies are being studied in numerous trials, and some already show significant benefits in survival rates. The development of new therapies to avert endocrine resistance is an urgent challenge in modern medicine. The following review will examine some promising therapeutic approaches.

Keywords: Advanced hormone receptor-positive breast cancer, endocrine resistance, CDK4/6 inhibitor, molecularly-targeted substances

INTRODUCTION

Breast cancer is the most commonly diagnosed type of cancer and the leading cause of cancer deaths in women worldwide[1]. The hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative subtype accounts for approximately 70% of cases[2]. Endocrine-based therapeutic strategies are the treatment of choice for this subtype unless there is a visceral crisis or other contraindication. Standard endocrine therapy for postmenopausal women includes selective estrogen receptor modulators (SERMs), selective estrogen receptor degraders (SERDs), non-steroidal aromatase inhibitors (NSAIs), and steroidal aromatase inhibitors[3]. The treatment algorithm has changed substantially since adding cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors to endocrine therapy showed significant outcome benefits. Nevertheless, resistance to endocrine therapies remains a persistent problem. Recent studies identified several mechanisms that lead to resistance to endocrine therapy; these studies identify novel therapeutic approaches that overcome resistance and improve efficacy.

Standard of care: CDK4/6 inhibition

Activation of CDK4/6 is central to regulating cell cycle progression and proliferation driven by estrogen[4,5]. The advent of CDK4/6 inhibitors targeting overactive CDK complexes has transformed the management of HR-positive and HER2-negative metastatic breast cancer. Three CDK4/6 inhibitors (palbociclib, ribociclib, abemaciclib) combined with endocrine therapy demonstrated meaningful clinical benefits[6-17]. Compared to standard chemotherapy, these combination therapies showed similar efficacy with a superior toxicity profile[6,7]. The phase III PEARL trial compared palbociclib plus endocrine therapy with capecitabine in postmenopausal women with aromatase inhibitor (AI)-resistant metastatic breast cancer to investigate differences in progression-free survival and safety profiles[6]. Palbociclib was combined with exemestane (cohort 1) or fulvestrant (cohort 2) and estrogen receptor 1 (ESR1) mutation status was assessed to prevent confounding by acquired endocrine resistance. There was no superiority of palbociclib plus endocrine therapy over capecitabine in both cohorts regarding progression-free survival[6]. On the other hand, significantly more high-grade adverse events were observed with capecitabine than with palbociclib plus endocrine therapy [Table 1]. As a consequence, the rate of treatment discontinuation was twice as high in the capecitabine arm[6].

One of the first promising signals of CDK4/6 inhibition came from the PALOMA-1/TRIO-18 trial published in 2015[8]. This phase II study showed that the addition of palbociclib to letrozole compared to letrozole alone significantly prolonged progression-free survival [20.2 months *vs.* 10.2 months; hazard ratio (HR) 0.488; $P = 0.0004$][8]. Since then, several phase III studies demonstrated the superiority of the addition of a CDK4/6 inhibitor to endocrine therapy compared to endocrine therapy alone regarding progression-free survival in first-line therapy for advanced HR-positive, HER2-negative breast cancer [Table 2][8-10,12,18].

Regarding overall survival, study results diverged in the first-line therapy setting. Of note, overall survival rates were assessed as secondary endpoints in the studies described below. This is consistent with standard practice, as survival rates in patients with HR-receptor-positive, HER2-negative breast cancer extend over several years, making progression-free survival the preferred primary endpoint[19]. This, in turn, affects the interpretation of overall survival data.

The MONALEESA-2 trial showed a significant overall survival benefit with ribociclib plus letrozole in the first-line therapy compared to letrozole alone[11]. The median overall survival was 63.9 months with ribociclib plus letrozole *vs.* 51.4 months with letrozole alone (HR 0.76; $P = 0.008$) after a median follow-up of 6.6 years[11]. The MONARCH-3 trial that investigated the efficacy of abemaciclib plus NSAI as first-line therapy compared to NSAI alone reported significantly longer progression-free survival and superior

Table 1. Summary of the most frequently reported adverse events of grade ≥ 3[6]

	Palbociclib plus exemestane *n* (%) *n* = 150	Palbociclib plus fulvestrant *n* (%) *n* = 149	Capecitabine *n* (%) *n* = 289
Neutropenia	86 (57.4)	83 (55.7)	16 (5.5)
Febrile neutropenia	2 (1.3)	1 (0.7)	4 (1.4)
Hand/foot syndrome	0	0	68 (23.5)
Diarrhea	2 (1.3)	2 (1.3)	22 (7.6)
Fatigue	2 (1.3)	1 (0.7)	16 (5.5)
Anemia	1 (0.7)	3 (2.0)	10 (3.5)
SAEs	24 (16.0)	19 (12.8)	63 (21.8)
SAEs related to therapy	6 (4.0)	5 (3.4)	30 (10.4)

n: Number of patients; SAEs: serious adverse events.

Table 2. Selected trials investigating CDK4/6 inhibitors as first-line therapy

Trial	*n*	Agent	Median PFS (mo.)	HR [95%CI]	Median OS (mo.)	HR [95%CI]
PALOMA-1 (phase II trial)[8,135]	165	Palbociclib + letrozole *vs.* letrozole	20.2 *vs.* 10.2	0.488 [0.319-0.748]	37.5 *vs.* 34.5	0.897 [0.623-1.294]
PALOMA-2 (phase III trial)[20,21]	666	Palbociclib + letrozole *vs.* letrozole	27.6 *vs.* 14.5	0.563 [0.461-0.687]	53.9 *vs.* 51.2	0.956 [0.777-1.177]
MONALEESA-2 (phase III trial)[11,18]	668	Ribociclib + letrozole *vs.* letrozole	25.3 *vs.* 16.0	0.568 [0.457-0.704]	63.9 *vs.* 51.4	0.76 [0.63-0.93]
MONALEESA-7 (phase III trial)[a][10,14]	672	Ribociclib + tamoxifen/NSAI *vs.* tamoxifen/NSAI All + GnRH	23.8 *vs.* 13.0	0.55 [0.44-0.69]	n.r. *vs.* 40.9	[37.8-n.r.]
MONARCH-3 (phase III trial)[12]	493	Abemaciclib + NSAI *vs.* NSAI	28.18 *vs.* 14.76	0.54 [0.418-0.698]	-	-

n: Number of patients; PFS: progression-free survival; mo.: months; HR: hazard ratio; CI: confidence interval; OS: overall survival; *vs.*: versus; NSAI: non-steroidal aromatase inhibitor; GnRH: Gonadotropin-releasing hormone; n.r.: not reached; [a]14% of patients received one line of prior chemotherapy for advanced disease in each study group.

objective response rates with the addition of abemaciclib; final overall survival data have not yet been reported[12].

The PALOMA-2 trial that investigated the efficacy of palbociclib combined with letrozole compared to letrozole alone reported significantly longer progression-free survival with palbociclib plus letrozole (27.6 *vs.* 14.5 months; HR 0.563; $P < 0.0001$) after a median follow-up of 38 months[20].

For these reasons, the overall survival rates presented at the Annual Meeting of the American Society of Clinical Oncology in 2022 were surprising. At a median follow-up of 90 months, the median overall survival with palbociclib plus letrozole was 53.9 months compared to 51.2 months with letrozole alone (HR 0.956; $P = 0.3378$)[21]. This result suggests that patients who received the combination therapy of palbociclib plus letrozole had numerically longer overall survival; however, the results were not statistically significant[21]. When interpreting these results, it is essential to note that no follow-up data were available (lost to follow-up or withdrawal of consent) for 21% of patients in the placebo-letrozole-arm *vs.* 13% of patients in the palbociclib-letrozole-arm[21]. Missing data were censored (assumed to be alive)[21]. According to the authors, the interpretation of the overall survival data of the PALOMA-2 trial was limited by this extensive and

disproportionate censoring of patients with missing survival data between the two treatment arms and the diversity of patients enrolled in the trial[22]. A post hoc sensitivity analysis was performed to counteract the effects of censoring by excluding patients with missing follow-up data. This operation resulted in a prolonged median overall survival of 51.6 months in the palbociclib-letrozole-arm *vs.* 44.6 months in the placebo-letrozole-arm [HR 0.869; confidence interval (CI) 0.706 to 1.069][21].

Preliminary insights from subgroup analyses with balanced amounts of missing follow-up data were presented at the American Society of Clinical Oncology Annual Meeting 2022[23]. They showed a benefit of palbociclib plus letrozole for patients with an Eastern Cooperative Oncology Group performance status of 1 or 2 (HR 0.801), disease-free interval of more than 12 months (HR 0.728), prior endocrine therapy (HR 0.801), and bone-alone disease (HR 0.712)[23]. Particularly outstanding was a median overall survival of 66.3 months for patients in the palbociclib-letrozole-arm with a disease-free interval of more than 12 months and a proportion of 10% of patients who continued to receive palbociclib plus letrozole after a median follow-up of 90 months[21]. To date, the final publication of these results is pending. Nevertheless, based on the available study results, the combination therapy of ribociclib plus endocrine therapy should be the preferred treatment regimen.

In second-line therapy, adding all three CDK4/6 inhibitors significantly prolonged progression-free and overall survival, as reported by the MONALEESA-3 trial for ribociclib, the MONARCH-2 trial for abemaciclib, and the PALOMA-3 trial for palbociclib [Table 3][15,21,24-26]. After a median follow-up of 44.8 months, the PALOMA-3 trial reported numerically longer overall survival for palbociclib plus fulvestrant compared to fulvestrant alone; however, the difference in the overall study group was not significant[24]. In patients who had sensitivity to prior endocrine therapy, the addition of palbociclib resulted in significantly longer overall survival (39.7 *vs.* 29.7 months; HR 0.72; CI: 0.55-0.94)[24]. After an extended follow-up of 73.3 months, the improvement in overall survival in the palbociclib-fulvestrant-arm was statistically significant in the entire study group[25].

Premenopausal women are treated like postmenopausal patients after adding a gonadotropin-releasing hormone agonist like goserelin to suppress ovarian function or bilateral ovariectomy[27]. The benefit of adding ribociclib to endocrine therapy and goserelin was examined separately for premenopausal women in the MONALEESA-7 trial[10,28]. The median progression-free survival was significantly prolonged to 23.8 months with ribociclib plus endocrine therapy compared to 13.0 months with endocrine therapy alone (HR 0.55; $P < 0.0001$)[10]. The recently published overall survival rates with an extended follow-up showed a statistically significant benefit in median overall survival of 58.7 months with ribociclib *vs.* 48.0 months with placebo (HR 0.76)[28]. This survival benefit was generally consistent in subgroup analyses, including patients younger than 40 years[28]. The analysis of the premenopausal subgroup in the MONARCH-2 trial was consistent with the improved progression-free and overall survival observed with abemaciclib plus fulvestrant in the intent-to-treat population[29].

Side effect management is essential when using a CDK4/6 inhibitor to improve patient adherence. Table 4 briefly overviews the most common grade 3 or 4 adverse events for each CDK4/6 inhibitor. A meta-analysis showed a significant increase in the rate of grade 3 and 4 adverse events with an addition of a CDK4/6 inhibitor to endocrine therapy compared to endocrine therapy alone, including neutropenia (HR 57.05; $P < 0.001$), leukopenia (HR 36.36; $P < 0.001$), and diarrhea (HR 4.97; $P < 0.001$)[17]. The choice of CDK4/6 inhibitor may also be based on the safety profile or dosing schedule. For example, the therapy with abemaciclib is associated with fewer hematologic side effects than the other CDK4/6 inhibitors; however,

Table 3. Selected trials investigating CDK4/6 inhibitors as ≥ second-line therapy

Trial	*n*	Agent	Median PFS (mo.)	HR [95% CI]	Median OS (mo.)	HR [95% CI]
PALOMA-3 (phase III trial)[25,136]	521	Palbociclib + fulvestrant *vs.* fulvestrant	9.5 *vs.* 4.6	0.46 [0.36- 0.59]	34.8 *vs.* 28.0	0.81 [0.65- 0.99]
MONARCH-2 (phase III trial)[26,137]	669	Abemaclclib + fulvestrant *vs.* fulvestrant	16.4 *vs.* 9.3	0.553 [0.449-0.681]	46.7 *vs.* 37.3	0.757 [0.606-0.945]
MONALEESA-3 (phase III trial)[138,139]	726	Ribociclib + fulvestrant *vs.* fulvestrant	20.5 *vs.* 12.8	0.593 [0.480-0.732]	53.7 *vs.* 41.5	0.73 [0.59-0.90]

n: Number of patients; PFS: progression-free survival; mo.: months; HR: hazard ratio; CI: confidence interval; OS: overall survival; *vs.*: versus.

Table 4. Common adverse events with CDK4/6 inhibitors; Modified from[13,136,140]

Adverse event	Abemaciclib plus ET		Palbociclib plus ET		Ribociclib plus ET	
Neutropenia	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**
Infection	45.1%	25.4%	81%	65%	74.3%	59.3%
Febrile	n.s.	n.s.	42%	2-3%	50.3%	4.2%
neutropenia	< 1%	n.s.	1%	1%	1.5%	n.s.
Anemia	30.1%	7.1%	28%	3%	18.6%	1.2%
AST elevation	14.2%	2.9%	7%	3%	15.0%	5.7%
ALT elevation	15.1%	5.1%	6%	2%	15.6%	9.3%
Diarrhea	84.6%	11.7%	21%	< 1%	35.0%	1.2%
Vomiting	27.7%	1.2%	17%	< 1%	29.3%	3.6%
Fatigue	40.5%	2.3%	39%	2%	36.5%	2.4%

ET: Endocrine therapy; ALT: alanine aminotransferase; AST: aspartate aminotransferase; n.s.: not specified.

diarrhea is reported more frequently. Notably, the combination of ribociclib and tamoxifen should be avoided because it prolongs QT intervals[11].

Based on these studies, international guidelines recommend the addition of a CDK4/6 inhibitor to endocrine therapy in the first-line treatment of metastatic HR-positive breast cancer[27]. The combination therapy is effective for newly diagnosed or recurrent advanced breast cancer in first- or second-line therapy and in cases of primary or secondary endocrine resistance[27]. However, the resistance to CDK4/6 inhibitors is a persistent challenge and a main of research[5,30-32]. It is not fully understood whether the development of resistance is associated with overcoming cell cycle inhibition or bypassing it by activating other signaling pathways instead[5]. In general, CDK4/6 form a complex with cyclin D to phosphorylate the retinoblastoma protein, leading to the release of transcription factors (especially E2F) that activate DNA transcription [Figure 1][33]. These mechanisms participate in carcinogenesis and the estrogen-driven proliferation of breast cancer cells[34,35]. One explanation for developing resistance to CDK4/6 inhibition is the overexpression of CDK4/6, in which CDK6 expression appears to play a dominant role[5,36]. In addition, several signaling pathways are associated with resistance to endocrine therapy and CDK4/6 inhibition. One of the most important is the PI3K/AKT/mTOR pathway (described below)[5]. Activation of the PI3K/AKT/mTOR pathway (for example, by acquired mutations or upregulation of the fibroblastic growth factor receptor pathway) is thought to stabilize the CDK4/6 complex leading to a reversal of CDK4/6 inhibition[37]. Although several mechanisms have been investigated, and several potential targets have been identified, overcoming resistance is a persistent challenge in clinical practice, and further clinical trials are urgently needed.

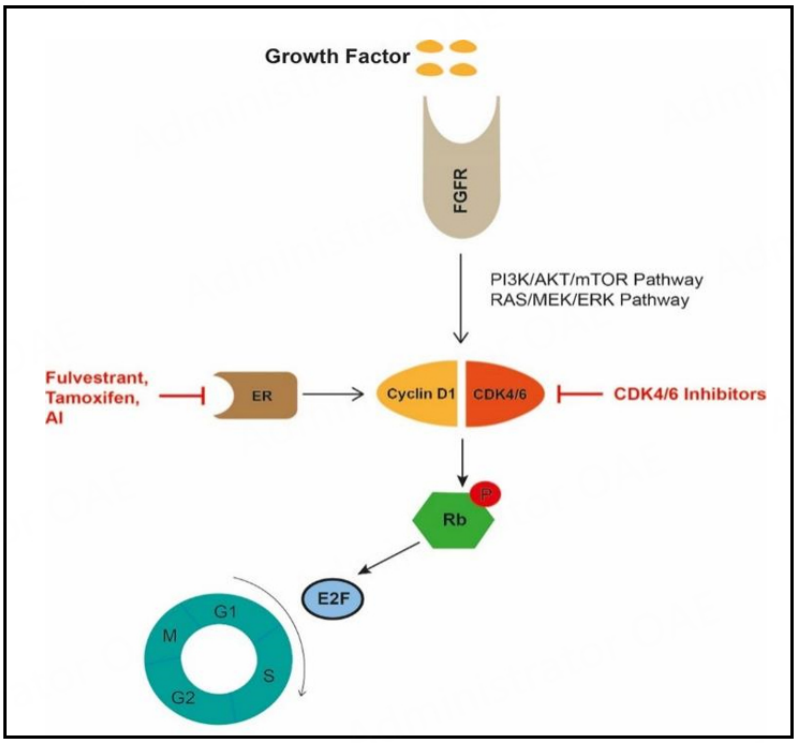

Figure 1. Mechanism of CDK4/6 inhibition; Modified from Li *et al.*, 2020[5]. FGFR: Fibroblast growth factor receptor; ER: estrogen receptor; AI: aromatase inhibitor; CDK4/6: cyclin-dependent kinases 4/6; P: phosphorylation; Rb: retinoblastoma protein; E2F: transcription factor E2F

PROMISING APPROACHES IN THE TREATMENT OF HR-POSITIVE BREAST CANCER

Retreatment with a CDK4/6 inhibitor after disease progression on CDK4/6 inhibition

Several studies investigated the benefit of further treatment with a CDK4/6 inhibitor after progression on the first-line treatment with a CDK4/6 inhibitor. Wander *et al.* investigated the efficacy of abemaciclib after disease progression on a prior CDK4/6 inhibitor (primarily palbociclib) in a retrospective cohort study[38]. This study was based on several unique pharmacological properties of abemaciclib compared to palbociclib or ribociclib and the demonstration of the efficacy of abemaciclib as a single-agent treatment in heavily pretreated patients in the phase II MONARCH-1 trial[39]. Most patients received abemaciclib as non-sequential therapy with ≥ 1 intervening regimen[38]. The survival rates reported by Wanderer *et al.* were similar to those seen in the MONARCH-1 trial, in which patients had not previously received a CDK4/6 inhibitor, and emphasized the efficacy of abemaciclib after progression on a prior CDK4/6 inhibitor[38,39]. Furthermore, several genomic alterations previously associated with CDK4/6 resistance were detected in patients with rapid progression on abemaciclib[38].

Kalinsky *et al.* reported positive results from the phase II MAINTAIN trial[40]. They showed a significant benefit in progression-free survival for patients who received ribociclib in combination with a switch of endocrine therapy after progression on a CDK4/6 inhibitor plus endocrine therapy compared to the group of patients who had a switch of endocrine therapy without adding ribociclib[40]. Unfortunately, it is impossible to deduce from the study design whether changing the CDK4/6 inhibitor to ribociclib alone (without a switch of endocrine therapy) would be sufficient to show a survival benefit. Several studies are currently underway to evaluate the value of subsequent treatment with a CDK4/6 inhibitor after prior progression on a CDK4/6 inhibitor. Although further studies are needed to draw definitive conclusions, the

positive signals are sufficient to conclude that a subset of patients benefits from retreatment with a CDK4/6 inhibitor.

Estrogen receptor 1 mutation

In HR-positive breast cancer, a central mechanism of acquired endocrine resistance, particularly to aromatase inhibitors, is a mutation in the drug target itself, such as a gain-of-function mutation in the ligand-binding domain of ESR1[41,42]. ESR1 mutations lead to ligand-independent estrogen receptor (ER) activity, promoting tumor growth and resistance to endocrine therapy[43]. The prevalence of ESR1 mutations depends on the prior duration of endocrine therapy and is detectable in 20%-40% of patients who have received an AI for metastatic breast cancer[42,44].

Interestingly, the mutation rate is much lower in the case of recurrent breast cancer and is less than 1% in endocrine therapy-naive patients, suggesting that ESR1 mutations are acquired mutations during AI treatment in the metastatic setting[42]. ESR1 mutations result in estrogen-independent activation of estrogen receptors and lead to resistance to AIs but not SERDs or SERMs[44-47]. ESR1 mutations usually occur with several other genomic alterations and are only partially responsible for the resistance developed[42]. Nevertheless, molecular testing can be helpful in case of cancer progression during endocrine therapy to predict resistance to AI therapy in the future.

There are several ways to detect ESR1 mutations. One is the non-invasive detection of circulating tumor DNA (ctDNA) in the patient's plasma[48-50]. Analysis of the SoFEA trial showed that patients with ESR1 mutations had significantly better progression-free survival with fulvestrant than exemestane (HR 0.52; $P = 0.02$), whereas patients without ESR1 mutation had similar survival rates after receiving either treatment (HR 1.07; $P = 0.77$)[46]. Concordant results were reported by a subgroup analysis of the PALOMA-3 trial[46,51]. ESR1 mutations were found in the serum of approximately 25% of patients; however, the survival benefit achieved by adding palbociclib to fulvestrant compared to placebo plus fulvestrant was independent of ESR1 mutation status[46].

There is preliminary evidence that ESR1 mutation status helps select further treatment options and monitor ongoing endocrine therapies. The PADA-1 trial was designed to demonstrate the efficacy of periodic monitoring of patients treated with palbociclib plus AI for emerging or rising ESR1 mutations in ctDNA to initiate an early treatment change to palbociclib plus fulvestrant, even before evidence of disease progression is apparent[52]. In the first step, 1017 patients were enrolled and treated with palbociclib plus an AI. After a median of 15.6 months, 172 patients with rising ESR1 mutations were randomized to continue therapy or to switch the therapy regime to palbociclib plus fulvestrant[53]. Preliminary data showed that the median progression-free survival in the palbociclib-AI-arm was 5.7 months *vs.* 11.9 months in the cohort that switched the treatment regime to fulvestrant plus palbociclib[53]. A preliminary analysis of safety outcomes confirmed the favorable safety profile of palbociclib in combination with any AI with or without a switch to fulvestrant[54]. Suppose these findings are confirmed in the final analysis and further studies. In that case, ESR1 mutation analysis will have clinical implications as an emerging biomarker for endocrine therapy decision-making in the future or could be used for early modification of therapy regimes to avoid expectable tumor progression even before it becomes apparent[52].

Oral selective ER degraders

Fulvestrant is a SERD that treats advanced HR-positive breast cancer; however, the application is limited to intramuscular injection. Fulvestrant antagonizes estrogen receptor alpha (ERα) and induces its degradation by binding the ligand-binding pocket[55,56]. Mutations in the ligand-binding-domain of ESR1 are responsible for resistance to AIs and reduce the potency of fulvestrant[43,57]. Efforts are currently underway to identify

next-generation, orally effective SERDs with improved efficacy and potency by optimizing the molecule's ability to saturate ERα, antagonizing its activity, and reducing its degradation[58].

Elacestrant is the first oral SERD that demonstrated better efficacy than endocrine therapy in patients with advanced HR-positive breast cancer[59]. The novel SERD functions by degrading ERα and inhibiting estradiol-dependent estrogen receptor-related gene transcription and tumor growth with improved pharmacological properties compared to fulvestrant[59-62]. The phase III EMERALD trial investigated the efficacy and safety of elacestrant in postmenopausal women with HR-positive, HER2-negative advanced or metastatic breast cancer who had progression after first- or second-line treatment with a combination of CDK4/6 inhibitor plus endocrine therapy[59]. Elacestrant was administered orally daily, and efficacy was compared with standard-of-care (SOC) endocrine therapy (fulvestrant/anastrozole/letrozole/exemestane monotherapy)[59]. Bidard *et al.* reported a significantly prolonged progression-free survival in patients treated with elacestrant compared to the SOC-cohort after a median follow-up of 15.1 months (HR 0.7; $P = 0.002$)[59]. Kaplan-Meier curves revealed six-month progression-free survival rates of 34.3% *vs.* 20.4% for the elacestrant *vs.* SOC arm[59]. The ESR1 mutation status was assessed using cell-free circulating DNA. Of the 477 patients, 47.8% had detectable ESR1 mutations[59]. ESR1-mutated patients had a significant improvement of progression-free survival with elacestrant compared with SOC endocrine therapy (HR 0.55; $P = 0.005$)[59]. In a subgroup analysis, the efficacy of elacestrant was also higher than in patients receiving fulvestrant as SOC[59]. An interim analysis of overall survival rates demonstrated hazard ratios of 0.75 (95%CI: 0.54-1.04; $P = 0.08$) in the overall population and 0.59 (95%CI: 0.36-0.96; $P = 0.03$) in the ESR1-mutated cohort[59]. The most common adverse events observed with elacestrant were nausea (35.0%), fatigue (19.0%), decreased appetite (14.8%), and arthralgia (14.3%), and grade 3 and 4 adverse events were reported in 27.0% of patients in the elacestrant arm *vs.* 20,5% in the SOC arm[59].

The EMERALD trial reported superior progression-free survival rates of elacestrant compared to fulvestrant in this selected population and might give a first hint that elacestrant could be an orally available alternative to the intramuscular injections of fulvestrant, especially for ESR1-mutated breast cancers[59].

Several promising oral SERDs are currently being investigated in numerous clinical trials. Table 5 provides a brief overview of selected clinical trials on oral SERDs, although not all studies have reported results. Recently, the first results of the phase II acelERA trial were reported. The study evaluated the efficacy and safety of giredestrant compared with endocrine therapy (fulvestrant or an AI) in patients with HR-positive, HER2-negative advanced breast cancer who progressed after one or two lines of systemic therapy[63]. Prior treatment with fulvestrant and a CDK4/6 inhibitor was allowed. The study did not meet its primary endpoint of investigator-assessed progression-free survival; however, monotherapy with giredestrant showed a numerical improvement of progression-free survival compared with endocrine therapy of physician's choice (5.6 months *vs.* 5.4 months; HR 0.81; $P = 0.1757$)[63]. A higher clinical benefit rate and objective response rate were reported with giredestrant[63]. In the cohort of patients with proven ESR1 mutation (39% of patients), the benefit in progression-free survival was even more pronounced (HR 0.60; $P = 0.0610$)[63]. By contrast, negative results were recently reported by the phase II AMEERA-3 trial[64].

Amcenestrant is another oral SERD that showed promising antitumor activity in phase I/II studies regardless of the ESR1 mutation status[65,66]. The efficacy of amcenestrant compared to endocrine therapy of physician's choice was investigated in the phase II AMEERA-3 trial in postmenopausal women with HR-positive, HER2-negative advanced breast cancer who received ≤ 2 prior lines of endocrine therapy and ≤ 1 prior chemotherapy or ≤ 1 targeted therapy for advanced disease[64]. Unfortunately, the study did not meet its primary endpoint, as progression-free survival was similar in both cohorts (median progression-free

Table 5. Selected trials investigating oral SERDs

Trial	Agent	Study population	Median PFS (mo.)
EMERALD (phase III trial)[59]	Elacestrant *vs.* standard of care	Patients with ER-positive, HER2-negative advanced breast cancer who received one or two lines of endocrine therapy; required pretreatment with a cyclin-dependent kinase 4/6 inhibitor, and no more than one prior line of chemotherapy	2.8 *vs.* 1.9; HR 0.70; *P* = 0.002 ESR1-mutant subgroup: 3.8 *vs.* 1.9; HR 0.55; *P* = 0.0005
AcelERA BC (phase II trial)[63]	Giredestrant *vs.* endocrine treatment of physician's choice (fulvestrant or aromatase inhibitor)	Post- and pre-/peri-menopausal women, or men with ER-positive, HER2-negative locally advanced or metastatic breast cancer who progressed after 1-2 lines of systemic therapy (≤ 1 targeted, ≤ 1 chemotherapy regimen, prior fulvestrant allowed)	5.6 *vs.* 5.4 HR 0.81 *P* = 0.18 ESR1-mutant subgroup: 5.3 *vs.* 3.5; HR 0.60; *P* = 0.0610
PersevERA (phase III trial)[141]	Giredestrant or letrozole plus palbociclib	Patients with ER-positive, HER2-negative advanced breast cancer who had no prior treatment for advanced disease	No results reported yet
AMEERA-5 (phase III trial)[67,68]	Amcenestrant or letrozole plus palbociclib	Patients with ER-positive, HER2-negative advanced breast cancer who have not received any prior systemic anticancer therapy for advanced disease	No results reported yet; trial discontinued based on the outcome of a prespecified interim analysis as the combination of amcenestrant plus palbociclib did not meet the prespecified boundary for continuation
AMEERA-3 (phase II trial)[64]	Amcenestrant *vs.* endocrine treatment of physician's choice (fulvestrant in 90% of cases)	Patients with ER-positive, HER2-negative metastatic or locally advanced breast cancer who received ≤ 2 prior lines of endocrine therapy and no more than one prior line of chemotherapy or targeted therapy for advanced breast cancer. Prior treatment with cyclin-dependent kinase 4/6 inhibitor was allowed.	3.6 *vs.* 3.7; HR 1.051; *P* = 0.6437
SERENA-4 (phase III trial)[142]	Camizestrant or anastrozole plus palbociclib	Patients with ER-positive, HER2-negative advanced breast cancer who have not received any systemic treatment for advanced disease	No results reported yet

PFS: Progression-free survival; mo.: months; *vs.*: versus; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; ESR1: estrogen receptor 1.

survival 3.6 *vs.* 3.7 months; HR 1.051)[64]. Amcenestrant as monotherapy showed no clinical benefit in patients that progressed during endocrine therapy. The reasons for the differences seen between the EMERALD trial and AMEERA-3 trial could be due to differences in the patient populations studied (e.g., ESR1 mutation rates or pretreatment with CDK4/6 inhibitors). In addition, the AMEERA-5 trial investigated the efficacy and safety of amcenestrant in combination with palbociclib, was recently discontinued due to negative results of a prespecified interim analysis showing that the treatment regime failed to meet the prespecified boundary over the control arm. In contrast, no new safety signals were observed[67,68]. A publication of the results is currently pending.

PI3K/AKT/mTOR pathway in HR-positive, HER2-negative advanced breast cancer

Many mechanisms (including alterations in intracellular signaling pathways critical for cell replication and survival) lead to the development of cancer itself, metastasis, or endocrine resistance. One essential pathway that is affected by mutations in more than 70% of HR-positive breast cancer cases is the

PI3K/AKT/mTOR pathway[37,69]. This pathway plays an essential role in developing endocrine resistances by stabilizing the CDK4/6 complex and reversing the effect of CDK4/6 inhibition[5,69,70]. Several interactions exist between the PI3K/AKT/mTOR pathway and the ER pathway[69]. Some of the interactions and therapeutic targets are shown in Figure 2. Targeting the PI3K/AKT/mTOR pathway is a promising approach to overcoming endocrine resistance and has been investigated by numerous studies, some of which have already shown a significant impact on survival rates[69]. Some selected studies are reviewed in more detail below.

mTOR inhibition

The most clinically relevant substance targeting the PI3K/AKT/mTOR pathway is everolimus, an mTOR complex 1 (mTORC1) inhibitor. The combination of everolimus and exemestane was used in clinical practice before CDK4/6 inhibitors emerged and remaines a mainstay in treating HR-positive breast cancer[71-76]. In the phase III BOLERO-2 trial, exemestane plus everolimus was compared to exemestane plus placebo in patients with HR-positive advanced breast cancer who had recurrence or progression during prior therapy with a NSAI[71]. The addition of everolimus to exemestane resulted in a clinically significant improvement in progression-free survival in the overall population and all subgroup analyses, including patients with visceral metastases[71-73]. In terms of median overall survival, the addition of everolimus did not result in statistically significant improvement (31.0 months for everolimus plus exemestane *vs.* 26.6 months for placebo plus exemestane; HR 0.89; log-rank $P = 0.14$)[74]. The most common grade 3 and 4 adverse events were stomatitis, anemia, hyperglycemia, fatigue, and pneumonitis, which occurred more frequently with everolimus than with placebo[71].

For confirmation, Im *et al.* initiated the EVEREXES trial involving 235 patients, including 199 from Asia[75]. The study investigated the efficacy of everolimus in combination with exemestane in postmenopausal women with HR-positive, HER2-negative advanced breast cancer previously treated with a NSAI[75]. Median progression-free survival in the Asian subgroup was similar to the survival in the overall population (9.3 months in both groups), with an overall response rate of 19.6%[75]. The reported data were consistent with previously published results of the BOLERO-2 trial, and no new safety signals were identified[72,75]. As CDK4/6 inhibitors had not been used when the study was conducted, subsequent studies have now examined the use of everolimus plus exemestane in patients previously treated with a CDK4/6 inhibitor.

Mo *et al.* reported a smaller progression-free survival benefit from treatment with everolimus plus exemestane in patients previously treated with a CDK4/6 inhibitor compared to patients who were not (3.8 months *vs.* 5.4 months)[77]. There was no significant difference in overall survival between the two groups[77]. In contrast, Cook *et al.* showed no influence of prior treatment with a CDK4/6 inhibitor on progression-free survival for patients treated with everolimus plus exemestane compared to patients without prior exposure (3.6 months *vs.* 4.2 months)[78]. In addition, they reported a numerical improvement in overall survival (15.6 months *vs.* 11.3 months) for patients previously treated with a CDK4/6 inhibitor[78]. The discussion of the efficacy of everolimus plus exemestane after treatment with a CDK4/6 inhibitor is accompanied by the discussion of the optimal treatment sequence. Jeong *et al.* investigated the efficacy and clinical outcome in dependence on the treatment sequence of everolimus and CDK4/6 inhibitors in a retrospective analysis[79]. The sequence of CDK4/6 inhibition plus endocrine therapy followed by everolimus plus exemestane was associated with a non-significant but numerical benefit in median progression-free and overall survival compared with the study arm that received everolimus plus exemestane followed by a CDK4/6 inhibitor plus endocrine therapy[79]. Considering tumor response rates, time to initiation of chemotherapy, and rates of treatment discontinuation, the study results showed a trend in favor of the sequence of CDK4/6 inhibition plus endocrine therapy, followed by everolimus plus exemestane[79]. In

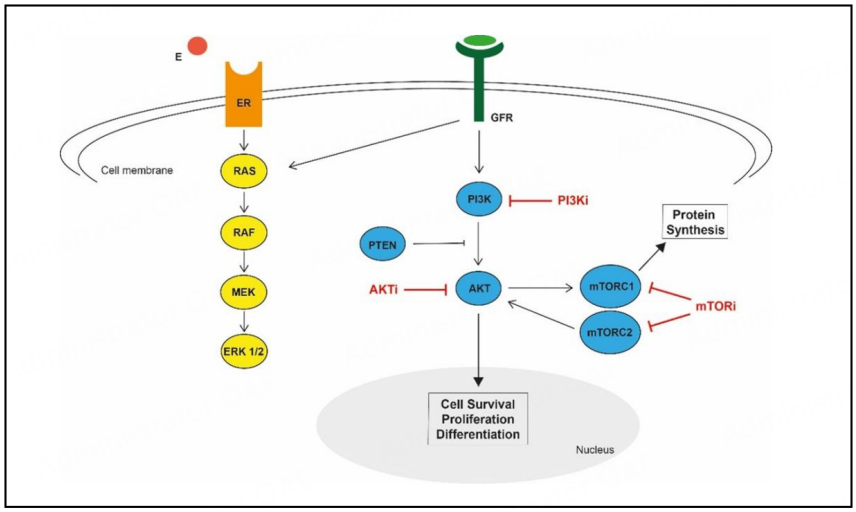

Figure 2. Targeting the PI3K/AKT/mTOR pathway; Modified from Du Rusquec et al., 2020[69]. E: Estrogen; ER: estrogen receptor; GFR: growth factor receptor; PI3K: phosphatidylinositol-3 kinase; PI3Ki: phosphatidylinositol-3 kinase inhibitor; AKT: protein kinase B; AKTi: AKT kinase inhibitor; PTEN: phosphatase and tensin homolog; mTORC1/2: mammalian target of rapamycin complex 1/2; mTORC1/2i: mammalian target of rapamycin complex 1/2 inhibitor.

conclusion, the authors suggested considering CDK4/6 inhibitor-based treatment regimes as an earlier treatment line as survival rates tend to favor the sequence of CDK4/6 inhibitor followed by everolimus[79]. Nevertheless, the results showed that subsequent therapy with everolimus plus exemestane after disease progression on a CDK4/6 inhibitor is justifiable, even if the additional benefit on survival rates is moderate.

There have been approaches with other mTOR inhibitors. Temsirolimus is an mTOR inhibitor that is selective for mTORC1. The HORIZON trial was designed to evaluate the clinical outcome and safety of adding temsirolimus to letrozole in AI-naive patients[80]. The study included 1112 postmenopausal patients with AI-naive, HR-positive advanced breast cancer[80]. Data analysis showed no improvement in progression-free survival in the temsirolimus-letrozole group compared to adding a placebo to letrozole[80]. On the other hand, significantly more adverse events, such as hyperglycemia, diarrhea, and stomatitis, were reported with temsirolimus[80]. Based on these results, it was speculated that the lack of improvement in survival was due to cancer cells in the metastatic setting not being exposed to endocrine therapy earlier. The cancer cells might not have depended on the PI3K/mTOR pathway and were thus insensitive to mTOR inhibition[81].

Another approach was taken with sapanisertib, a selective mTOR inhibitor with dual specificity against mTORC1 and mTOR complex 2[82,83]. Preclinical studies supported the theory that dual inhibition of both protein complexes might significantly suppress cancer cell proliferation[84,85]. Based on this assumption, García-Sáenz *et al.* hypothesized that dual inhibition with sapanisertib may restore the sensitivity of the cancer cell to endocrine therapies in patients with HR-positive breast cancer[86]. The phase II trial compared the daily or weekly application of sapanisertib plus fulvestrant with fulvestrant alone in postmenopausal women whose tumors had progressed during therapy with an AI[86]. Median progression-free survival was numerically longer in patients treated with sapanisertib plus fulvestrant (daily use: 7.2 months; weekly use: 5.6 months) than with fulvestrant alone (3.5 months), with the most significant improvement in patients who had previously received a CDK4/6 inhibitor[86]. However, there was no statistical significance for daily or weekly application, and the rates of reported adverse events were higher in either arm of combination therapy (32% and 36%) compared with fulvestrant alone (4%)[86]. Based on these results, the authors did not

support further development of sapanisertib using these dosing schedules[86].

PI3K inhibition

Approximately 40% of patients with HR-positive, HER2-negative breast cancer have activating mutations in the gene encoding the alpha catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA), resulting in hyperactivation of the alpha isoform of phosphatidylinositol 3-kinase (PI3Kα)[87,88]. Several preclinical studies have demonstrated interactions between the ER and PI3K signaling pathways, as inhibition of PI3K leads to the upregulation of ER signaling[89]. In addition, PI3K inhibition enhances ER function and dependence in HR-positive breast cancer[89,90]. First-generation PI3K inhibitors, also known as pan-PI3K inhibitors, target all four isoforms of class I PI3K. The most studied pan-PI3K inhibitors for breast cancer treatment are buparlisib and pictilisib[91,92]. Unselective inhibition of all PI3K isoforms is accompanied by a high incidence of adverse events and leads to high treatment discontinuation rates, as shown in Table 6. As a result, these pan-PI3K inhibitors were not further investigated in subsequent trials. Over time, isoform-specific PI3K inhibitors emerged and opened new possibilities. The most important of these so far is alpelisib, a PI3Kα-specific inhibitor. The SOLAR-1 trial investigated the efficacy of alpelisib in patients with HR-positive, HER2-negative advanced breast cancer who had previously received endocrine therapy[93]. The phase III trial compared the combination therapy of alpelisib plus fulvestrant with placebo plus fulvestrant. In the cohort of patients with PIK3CA-mutated cancer, a prolongation of progression-free survival of 11.0 months was reported in the alpelisib-fulvestrant group compared to 5.7 months in the control group (HR 0.65; $P < 0.001$)[93]. A clinically relevant treatment benefit was not observed for alpelisib-fulvestrant in the cohort without PIK3CA-mutated cancer[93]. PIK3CA mutation testing does not necessarily need to be performed on tumor tissue, as a preliminary analysis of ctDNA-based results showed similar effects[93,94]. Alpelisib-related adverse events of grade 3 or 4 included hyperglycemia, diarrhea, or maculopapular rash, and the percentage of patients that discontinued alpelisib due to adverse effects was 25% (compared to 4.2% in the placebo-fulvestrant-group)[93]. The latter demonstrates the importance of good adverse event management during alpelisib therapy. The final analysis of overall survival in the SOLAR-1 trial did not reach the prespecified boundaries for statistical significance but showed a numerical improvement of 39.3 months compared with 31.4 months when alpelisib *vs.* placebo was added to fulvestrant (HR 0.86; $P = 0.15$)[95]. To evaluate the clinical utility of alpelisib in the treatment algorithm of breast cancer, the BYLieve trial was designed to investigate the efficacy of alpelisib in patients who had experienced disease progression during or after treatment with a CDK4/6 inhibitor plus AI[96]. The results supported the clinical benefit of alpelisib following treatment with a CDK4/6 inhibitor[96,97]. This leads to the conclusion that in case of disease progression during treatment with a CDK4/6 inhibitor plus endocrine therapy, the patient should be evaluated for a PIK3CA mutation.

Recently, another intensively studied agent was the β-isoform-sparing pan-PI3K inhibitor taselisib. The phase III SANDPIPER trial investigated the efficacy and safety of taselisib in combination with fulvestrant in women with disease progression or recurrence during or after treatment with an AI[90]. A statistically significant but small prolongation of progression-free survival was reported in patients with PIK3CA-mutant tumors in the taselisib-fulvestrant arm compared with the placebo-fulvestrant arm (7.4 months *vs.* 5.4 months; HR 0.70; $P = 0.0037$)[90]. The proportion of patients who experienced adverse events of grade ≥ 3 was 49.5% in the taselisib-fulvestrant arm *vs.* 16.4% in the placebo-fulvestrant arm, with diarrhea and hyperglycemia being the most frequently reported events[90]. Serious adverse events occurred in as many as 32% of patients receiving taselisib (*vs.* 16.4% in the placebo arm)[90]. In total, 16.8% of patients in the taselisib arm discontinued treatment, compared with 8.9% in the placebo arm[90]. Given the safety profile and modest clinical benefit, the authors concluded that taselisib had no clinical benefit despite meeting the primary endpoint[90].

Table 6. Selected PI3K inhibitors in HR-positive, HER2-negative breast cancer

Trial	Agent	Isoform-specific inhibition of PI3K	Median PFS (mo.)	SAE (%)	Discontinuation rate (%)
BELLE-2 (phase III trial)[91]	Buparlisib or placebo plus fulvestrant	Pan-PI3K inhibition (α, β, δ, γ)	Total population: 6.9 *vs.* 5.0; HR 0.78; *P* = 0.00021 PIK3CA mutant ctDNA: 4.6 *vs.* 1.5; HR 0.58; *P* = 0.036	23 *vs.* 16	39 *vs.* 5.0
FERGI (phase II trial)[92]	Pictilisib or placebo plus fulvestrant	Pan-PI3K inhibition	6.5 *vs.* 5.1 HR 0.73 *P* = 0.268	16 *vs.* 1	34 *vs.* 15
SOLAR-1 (phase III trial)[93]	Alpelisib or placebo plus fulvestrant	α-specific	11.0 *vs.* 5.7; HR 0.65 *P* < 0.001	34.9 *vs.* 16.7	25.0 *vs.* 4.2
SANDPIPER (phase III trial)[90]	Taselisib or placebo plus fulvestrant	β-isoform-sparing pan-PI3K inhibitor	7.4 *vs.* 5.4; HR 0.7; *P* = 0.0037	32 *vs.* 8.9	16.8 *vs.* 2.3

PI3K: Phosphoinositide 3 kinase; HR: hormone receptor; HER2: human epidermal growth factor receptor 2; PFS: progression-free survival; mo.: months; SAE: serious adverse event; *vs.*: versus; HR: hazard ratio; PIK3CA: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; ctDNA: circulation tumor DNA.

In summary, the PI3K pathway is involved in many mechanisms, including carcinogenesis, proliferation, and development of resistance in HR-positive breast cancer. Currently, alpelisib is the only available agent for patients with PIK3CA mutation. Although several PI3K inhibitors have been developed and are under evaluation in various stages of clinical trials, many agents have demonstrated only modest clinical benefit, with high rates of high-grade adverse events and treatment discontinuation rates. This is partly due to the inhibition of the p110alpha subunit of PI3K, which is physiologically involved in glucose metabolism and, when inhibited, responsible for hyperglycemia[98]. Nevertheless, inhibition of the PI3K pathway remains a highly interesting research target, mainly because the clinical application of PI3K pathway inhibition is not limited to HR-positive breast cancer. A deeper understanding of the secondary effects of PI3K inhibition and management of adverse events is necessary to improve PI3K-specific treatment. Furthermore, several studies investigated the efficacy of combined inhibition of the PI3K/AKT/mTOR pathway, CDK4/6, and ER signaling pathway to overcome the acquired resistance to CDK4/6 inhibition[99,100].

Michaloglou *et al.* demonstrated that combining an mTOR inhibitor with a CDK4/6 inhibitor resulted in a more durable growth arrest of cancer cells and delayed the development of resistance *in vitro*[99]. Similarly, several studies confirmed that the triple combination of a PI3K inhibitor, CDK4/6 inhibitor, and endocrine therapy could reverse endocrine resistance *in vitro*[30,100]. In the future, a combination of these therapies could lead to a long-lasting therapeutic effect. Clinical trials are ongoing.

AKT inhibition

Another frequently mutated tumor-suppressor gene is phosphatase and tensin homolog (PTEN) which functions as a negative regulator of the PI3K/AKT/PTEN pathway. In the HR-positive metastatic breast cancer subgroup, approximately 5%-10% of patients harbor a somatic mutation[101,102]. The mutation leads to loss of function and is associated with a poor prognosis and resistance to endocrine therapy[103-105]. Less frequently, alteration of the PI3K/AKT pathway is caused by AKT substitution or amplification[87,106]. Because a high proportion of HR-positive breast cancers exhibit hyperactivation of the PI3K/AKT pathway, several AKT kinase inhibitors have been investigated in clinical trials. The most promising AKT kinase inhibitor to date is capivasertib. Capivasertib is an oral pan-AKT kinase inhibitor investigated in several

studies and demonstrated antitumor activity[107-110]. The efficacy of capivasertib in combination with fulvestrant was tested in patients with PTEN-mutant HR-positive metastatic breast cancer in a phase I multipart expansion study by Smyth *et al.*[108]. The results showed a clinical benefit rate 17% in fulvestrant-naive and 42% in fulvestrant-pretreated patients after 24 weeks and an objective response rate of 8% *vs.* 21%[108]. Co-mutations occurred in PIK3CA, ESR1, and TP53, with a clonal dominance of PTEN in most patients[108]. In conclusion, the study reported the efficacy and antitumor activity of capivasertib plus fulvestrant with an acceptable safety profile in this heavily pretreated cohort[108]. The results showed slightly better efficacy in fulvestrant-pretreated patients, but notable phenotypic and genomic differences were observed between the cohorts[108].

The FAKTION trial is another study evaluating the AKT kinase inhibitor capivasertib. The phase II study investigated the effect of adding capivasertib to fulvestrant on progression-free survival in patients with AI-resistant advanced breast cancer[109]. Median progression-free survival was significantly longer in the capivasertib plus fulvestrant group than in the placebo-fulvestrant group (10.3 *vs.* 4.8 months; HR 0.58; $P = 0.0044$)[109]. Furthermore, adding capivasertib significantly prolonged overall survival to 29.3 months compared to 23.4 months in the placebo group (HR 0.66; two-sided $P = 0.035$)[111]. A second analysis was performed with patients identified as expanded PI3K/AKT/PTEN pathway-altered subgroup by an expanded biomarker panel. Survival rates in this specific subgroup showed even better results: Median progression-free survival was 12.8 months in the capivasertib arm *vs.* 4.6 months in the placebo arm (HR 0.44; $P = 0.0014$). Furthermore, median overall survival was 38.9 months with capivasertib plus fulvestrant *vs.* 20.0 months in the placebo arm (HR 0.46; $P = 0.0047$)[111]. In comparison, there was no statistical significance in survival rates between the treatment arms in the expanded pathway non-altered subgroup[111]. Adverse events such as hypertension, diarrhea, and fatigue were more common with capivasertib, and serious adverse events occurred only in the capivasertib arm[109]. These results once again highlight the utility of biomarker testing. Currently, the efficacy of capivasertib plus fulvestrant is being further evaluated by the phase III CAPItello-291 trial. The results of this trial will not only provide further insights into the efficacy of capivasertib but also demonstrate the efficacy in patients previously treated with a CDK4/6 inhibitor, as the latter was an exclusion criterion in the FAKTION trial.

Pathogenic variants in DNA-repair-related genes

In addition to identifying mechanisms leading to acquired endocrine resistance, the detection of germline mutations has become a mainstay of individualized tumor therapy. It is increasingly becoming possible to perform comprehensive genomic tumor profiling using a variety of multigene assays that allow the detection of potentially treatable genetic alterations[112]. In unselected populations of breast cancer patients, approximately five percent carry a germline BReast CAncer (BRCA) gene mutation[113,114]. A mutation in the BRCA 1 gene is known to predispose to triple-negative breast cancer, while patients with BRCA 2 mutation are most likely to develop HR-positive breast cancer[115,116]. Germline testing of patients with HER2-negative metastatic breast cancer for a BRCA 1/2 mutation as a predictive biomarker for the efficacy of poly(adenosine diphosphate-ribose) polymerase (PARP) inhibition is now part of the mandatory diagnostic workup in metastatic breast cancer. PARP inhibitors were initially used in treating ovarian cancer, where PARP inhibition has become an integral part of the treatment algorithm[117-119]. Recently, PARP inhibition has also been shown to have a promising effect and clinically meaningful benefit in patients with metastatic breast cancer and germline BRCA mutation[120-123]. In the OlympiAD trial, olaparib monotherapy provided a significant benefit over standard therapy in patients with HER2-negative metastatic breast cancer who had not received more than two prior chemotherapy regimens for metastatic disease[120]. Median progression-free survival was significantly longer (7.0 months *vs.* 4.2 months; $P < 0.001$), and the response rate was significantly higher (59.9% *vs.* 28.8%) compared to the standard therapy group[120]. At the same time, fewer high-grade adverse events were observed with olaparib monotherapy, resulting in a lower treatment

discontinuation rate due to toxic effects[120]. Meanwhile, another study also demonstrated an overall survival benefit with olaparib monotherapy in patients receiving olaparib as first-line therapy[121]. Similar positive results were reported by the EMBRACA trial, which investigated the efficacy of talazoparib, another PARP inhibitor[122]. Talazoparib provided a significant benefit over standard chemotherapy in terms of progression-free survival (8.6 months *vs.* 5.6 months; $P < 0.001$) and patient-reported outcomes[122].

One of the selection criteria of the described studies was a proven germline mutation. However, monotherapy with olaparib also showed positive effects in the presence of a somatic BRCA mutation in a phase II trial by Tung *et al.*[124]. The same study investigated the effect of olaparib in patients with metastatic breast cancer and mutations in homologous recombination-related genes other than BRCA 1/2. However, it confirmed an improvement in progression-free survival only in patients with germline "partner and localizer of BRCA 2" (PALB2) mutation[124]. These results may indicate that the population of breast cancer patients who could benefit from PARP inhibition could be expanded through further studies.

A recently published, retrospective real-world study by Bruno *et al.* investigated the impact of an existing germline pathogenic variant in a DNA repair-related gene on the therapeutic efficacy of a CDK4/6 inhibitor[125]. Reported pathogenic variants were germline mutations in the BRCA 1/2, Ataxia Telangiectasia Mutated, and Checkpoint kinase 2 genes[125]. A proven germline mutation was associated with a shorter median progression-free survival (10.2 months) compared with patients without these mutations (15.6 months) or nontested patients (17.6 months)[125]. The multivariable analysis revealed that mutation status was an independent prognostic factor associated with shorter progression-free and overall survival in patients receiving CDK4/6 inhibitors[125]. This highlights the need for genetic testing to better select the therapy strategy for specific patient populations.

Histone deacetylase inhibition - an example of an epigenetic approach

Unlike genetic mutations, epigenetic alterations are not due to mutations in the primary DNA sequence but cause changes in gene expression. The role of epigenetics in tumor progression and the development of endocrine resistance in HR-positive breast cancer is an emerging field of clinical investigation[126]. There are first promising approaches to reverse to effects of epigenetic alterations by epigenetic modifiers, such as histone deacetylase (HDAC) inhibitors[127]. Entinostat is an orally administered HDAC inhibitor currently under investigation as it has shown potential antiproliferative activity in breast cancer cells[128]. On the pharmacological level, it functions by inhibiting the enzyme histone deacetylase, which is thought to play an essential role in regulating gene expression through epigenetic modifications[127]. HDAC inhibition leads to the downregulation of estrogen-independent signaling pathways and causes a normalization of ER levels[129,130]. Available data show controversial results regarding the impact of entinostat on survival rates[127,131,132]. A phase II study investigated the influence of the addition of entinostat *vs.* placebo once a week to the daily application of exemestane in postmenopausal women with HR-positive advanced breast cancer who had tumor progression on a NSAI[133]. The addition of entinostat improved median progression-free survival to 4.3 months *vs.* 2.3 months with placebo (HR 0.73; one-sided $P = 0.055$; two-sided $P = 0.11$) and median overall survival to 28.1 months *vs.* 19.8 months with placebo (HR 0.59; $P = 0.036$)[133]. Entinostat was generally well tolerated, with leading adverse events of grade 3 or 4 being fatigue and neutropenia[133]. A phase III trial with a Chinese patient cohort with advanced HR-positive breast cancer also reported a positive impact of entinostat[132]. Analysis of 354 enrolled patients showed an improved progression-free survival of 6.32 months in the exemestane-entinostat-arm compared to 3.72 months in the exemestane-placebo-arm (HR 0.74; $P < 0.001$)[132]. Reported adverse events included neutropenia, thrombocytopenia, and leucopenia, all of them significantly more often in patients treated with entinostat[132]. In contrast, another randomized phase III trial with similar inclusion criteria reported no improvement in progression-free (3.3 *vs.* 3.1 months; HR 0.87; $P = 0.03$) or overall survival (23.4 *vs.* 21.7 months; HR 0.99; $P = 0.94$),

although the pharmacodynamic analysis confirmed the target inhibition in the entinostat-treated cohort[131]. Nevertheless, epigenetic modifiers open a new field of research in treating HR-positive breast cancer that will certainly gain importance in the future. Furthermore, preclinical data provide evidence that combining entinostat with palbociclib enhances the antitumoral activity of both drugs[134]. Further clinical studies on this novel and promising substance are still pending.

CONCLUSION

Endocrine therapeutic strategies are currently being improved and expanded by adding molecularly-targeted substances. These play an increasingly important role in managing advanced hormone receptor-positive breast cancer, particularly given the central problem of resistance to endocrine therapy. The search for biomarkers must be intensively pursued to individualize cancer therapies further. CDK4/6 inhibitors, in addition to endocrine therapy, have already become the standard of care in the first-line treatment of HR-positive advanced breast cancer. However, several novel promising substances (e.g., oral SERDs or PI3K inhibitors) are also being investigated in clinical trials, so an expansion of therapy options can be expected shortly. Given the rapid scientific progress, knowledge and management of adverse events are critical, as the new targeted agents are associated with side effects that differ significantly from endocrine therapy alone.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Droste A, Schmidt M

Availability of data and materials

Not applicable.

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None.

Conflicts of interest

Marcus Schmidt has received personal fees from AstraZeneca, BioNTech, Daiichi Sankyo, Eisai, Lilly, MSD, Novartis, Pantarhei Bioscience, Pfizer, Pierre Fabre, Roche, and SeaGen. His institution has received research funding from AstraZeneca, BioNTech, Eisai, Genentech, German Breast Group, Novartis, Palleos, Pantarhei Bioscience, Pierre Fabre, and SeaGen. In addition, he has a patent for EP 2390370 B1 and a patent for EP 2951317 B1 issued. Annika Droste reports no conflict of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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