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Tumour Review

Overcoming Resistance to CDK4/6 inhibitors in Hormone Receptor positive, HER2 negative breast cancer: Innovative Combinations and Emerging Strategies

Federica Giugliano [a,b,c], Carmine De Angelis [d], Barbara Pistilli [b,e], Giulia Viale [f,g], Giampaolo Bianchini [f,g], Mario Giuliano [h], Luca Malorni [i], Beatrice Taurelli Salimbeni [j], Angela Esposito [j], Antonio Giordano [k], Timothy A. Yap [l], Giuseppe Curigliano [c,j], Carmen Criscitiello [c,j,*]

[a] INSERM U981, Gustave Roussy, Villejuif, France
[b] Department of Medical Oncology, Gustave Roussy, Villejuif, France
[c] Department of Oncology and Hematology-Oncology, University of Milano, Milan, Italy
[d] Clinical and Translational Oncology, Scuola Superiore Meridionale Via Mezzocannone, 4 80134 Naples, Italy
[e] INSERM U1279, Gustave Roussy, Villejuif, France
[f] Università Vita-Salute San Raffaele, Milan, Italy
[g] Department of Medical Oncology, IRCCS Ospedale San Raffaele, Milan, Italy
[h] Department of Clinical Medicine and Surgery, University Federico II, Naples, Italy
[i] Translational Research Unit, Department of Oncology, Hospital of Prato, Azienda USL Toscana Centro, Prato, Italy
[j] European Institute of Oncology, IRCCS, Milan, Italy
[k] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA
[l] Department of Investigational Cancer Therapeutics, Division of Cancer Medicine, The University of Texas MD Anderson Cancer, Houston, USA

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ABSTRACT

Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in combination with endocrine therapy (ET) improve outcomes patients affected by metastatic and early-stage hormone receptor-positive, HER2-negative breast cancer. However, approximately 20% of these tumors exhibit intrinsic resistance to such therapies, and most develop acquired resistance mechanisms that drive progression. Biomarker analyses of biological samples from patients treated with CDK4/6i plus ET have identified potential targets for therapeutic combinations. In this review, we discuss the mechanisms of action and resistance to CDK4/6i, providing a comprehensive overview of emerging efficacy and safety data, biomarker-driven strategies, and ongoing clinical trials. Finally, we delineate key research priorities aimed at guiding the development of innovative therapeutic combinations.

Introduction

Hormone receptor-positive (HR +), human epidermal growth factor 2 negative (HER2-) breast cancer (BC) is the most commonly diagnosed type of BC, accounting for approximately 70 % of cases [1,2]. The therapeutic landscape for this subtype has been significantly

Abbreviations: AE, Adverse event; AI, Aromatase inhibitors; BC, Breast cancer; CBR, Clinical benefit rate; CDK, Cyclin-dependent kinase; CDK4/6i, CDK4/6 inhibitors; CERAN, Complete estrogen receptor covalent antagonists; ctDNA, Circulating tumor DNA; ER, Estrogen receptor; ET, Endocrine therapy; FDA, U.S. Food and Drug Admnistration; G, Grade; HER2, Human epidermial receptor 2; HER2-, HER2-negative; HR, Hazard Ratio; HR+, Hormone receptor positive; ICI, Immune checkpoint inhibitors; iDFS, Invasive Disease-Free Survival; mBC, Metastatic breast cancer; OS, Overall survival; PFS, Progression free survival; PROTAC, Targeted proteolysis chimera technology; mPFS, Median Progression Free survival; Rb1, Retinoblastoma protein 1; SERCA, Selective estrogen receptor covalent antagonists; SERD, Selective ER degrader; TILs, Tumor infiltrating lymphocites; TME, Tumor microenvironment; VEGF(R), Vascular endothelial growth factor (receptor); 95% CI, 95% Confidence Interval.

* Corresponding author at: Division of Early Drug Development for Innovative Therapy, IEO, European Institute of Oncology IRCCS, Via Ripamonti 435, 20141 Milan, Italy.
E-mail address: carmen.criscitiello@ieo.it (C. Criscitiello).

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transformed by the introduction of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i), such as palbociclib, ribociclib and abemaciclib [3]. Mechanistically, CDK4/6i synergize with endocrine therapy (ET) to disrupt aberrant cell cycle progression, inducing cellular senescence and eventual cell death [4]. In combination with aromatase inhibitors (AI) or fulvestrant, CDK4/6i have become a cornerstone of treatment across various clinical settings, improving outcomes in both metastatic setting and early-stage disease. In the metastatic setting, CDK4/6i represents the standard of care for first- or subsequent-line treatment for patients with HR+/HER2- metastatic BC (mBC) in combination with AI or fulvestrant in both Europe and the U.S.A. [5,6]. In the early therapeutic setting, the MonarchE and NATALEE trials have demonstrated that the addition of a CDK4/6i (abemaciclib and ribociclib) to standard adjuvant ET improves local and distant recurrence rate in HR+/HER2- BC with a higher risk of relapse [7–12]. Novel combinations exploiting CDK4/6i, innovative ET, and new pharmaceutical compounds targeting resistance pathways have recently been explored. In this review, we provide a comprehensive overview of the mechanisms of action and resistance to CDK4/6i. Additionally, we examined the available clinical evidence and ongoing trials exploring innovative therapeutic combinations with CDK4/6i, elucidating their evolving role in the management of HR+/HER2- BC.

Role of CDK4/6 proteins in breast cancer biology

Uncontrolled cellular proliferation, driven by the dysregulation of mechanisms governing cell division, is a hallmark of cancer [13]. The normal cell cycle is tightly regulated by a series of checkpoints and processes that ensure orderly progression through cell division (Fig. 1).

Cyclin-dependent kinases (CDKs), which are activated by binding to specific proteins called cyclins, play a pivotal role in modulating these transitions. CDK4 and CDK6 are structurally and functionally similar kinases that govern the G1 phase restriction point [3]. In the quiescent (G0) and early G1 phases, D-type cyclins (cyclin D1, D2, and D3) are expressed at low levels. As cyclin D levels rise, CDK4/6 forms complexes with cyclin D, which phosphorylate the retinoblastoma protein (Rb1), a key regulator of cell cycle progression. Unphosphorylated Rb1 typically represses cell cycle progression by binding to E2F transcription factors, which regulate genes essential for S-phase entry. Phosphorylation by cyclin D–CDK4/6 reduces Rb1′s affinity for E2F, enabling partial activation of E2F and initiating S-phase gene transcription, including cyclin E. Cyclin E forms complexes with CDK2, further phosphorylating Rb1 in a positive feedback loop, thereby fully activating E2F and driving the transition to S-phase [3].

Two families of inhibitory proteins modulate CDK4/6 activity: INK and CIP/KIP. Among the INK family, p16 (encoded by *CDKN2A* gene) is the most extensively characterized and is upregulated in response to Rb1 loss, acting as a negative feedback mechanism to halt cell cycle progression [3]. Specifically, the p16 protein forms a complex with CDK4 or CDK6 proteins, in which the binding affinity for cyclin D is reduced, thereby preventing Rb1 inhibition. In the CIP/KIP family, p27 binds to the CDK4/6–cyclin D complex, forming a trimeric structure that modulates CDK4/6 activity [3].

Effective inhibition of CDK4/6 signaling in cancer depends on a functional cyclin D–CDK4/6–Rb1 pathway and cell-specific reliance on CDK4/6 for progression through the G1 restriction point. In preclinical mouse models, CDK4 or CDK6 knockout is non-lethal, probably due to compensatory mechanisms between the two proteins [14]. Simultaneous knockout of both CDK4 and CDK6 results in embryonic lethality due to severe anemia, although non-hematopoietic cells remain unaffected, with CDK2 taking over their proliferative roles [3]. In a different BC model, *CCND1* ablation severely impaired tumor formation [15]. These data suggest that, in contrast to normal cell growth, BC displays a strong dependency on the cyclin D-CDK4/6-Rb1 pathway, with CDK4 and 6 representing critical targets for BC treatment.

Indeed, HR + BC has been shown to be dependent on CDK4/6 pathway for cell cycle progression. In particular, cyclin D1 is frequently overexpressed in HR + BC, with approximately 13 % of primary cases exhibiting amplification of *CCND1* gene, which encodes cyclin D1 [16]. Cases of *CDKN2* inactivation have been described, while *RB1* loss of function is uncommon (about 3 % of cases) [17]. Crosstalk between cyclin D1 and the estrogen receptor (ER) has also been described.[18] ER activity itself regulates *CCND1* expression, whereas cyclin D1 can activate ER signaling independent of estrogen. CDK4 is the dominant kinase in ER + BC cells, driving proliferation, and high CDK4 levels are associated with endocrine resistance [19]. Conversely, CDK6 activity is more relevant in bone marrow progenitor cells. This complex relationship likely underpins the dependence of ER + BC cells on CDK4/6 activity for cell cycle progression [4].

CDK4/6 inhibitors in breast cancer therapy

The three CDK4/6i approved for clinical use globally, palbociclib, ribociclib, and abemaciclib, along with dalpiciclib, which is approved exclusively in China, are designed to selectively target CDK4/6, albeit with varying degrees of activity against other CDKs (Table 1) [4]. Palbociclib exhibits comparable affinity for both CDK4 and CDK6, whereas ribociclib and abemaciclib demonstrate greater potency as CDK4

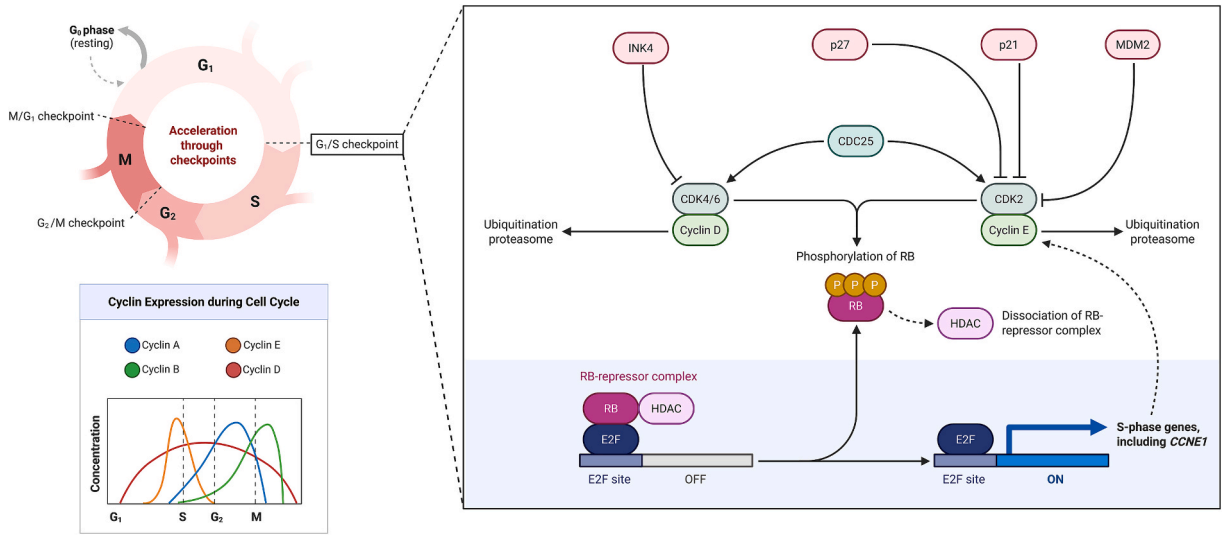

Fig. 1. Simplified overview of the role of CDK4/6 in the G1/S checkpoint. Created using Biorender.com.

Table 1
Differences among approved CDK4/6 inhibitors. Adverse events for CDK4/6 inhibitors plus endocrine therapy in first-line setting in aromatase inhibitors naïve hormone receptor positive, HER2-negative breast cancer. In brackets rate of adverse events with grade major or equal to 3.

Name	Half-life (hours)	CDK Affinity			CNS penetration	Adverse events							Ref
		CDK4	CDK6	CDK2		Neutropenia	Leukopenia	Nausea	ALT increase	Fatigue	Diarrhea	Other	
Palbociclib	29	++	++	–	–	79.5 % (66.5 %)	39 % (24.8 %)	35.1 % (0.2 %)	–	37.4 (1.8 %)	–	–	PALOMA-2[109]
Ribociclib	32	++	+	–	–	41.3 (21.1 %)	20.8 % (7.6 %)	38.5 % (0.9 %)	15.6 % (9.8 %)	40.1 % (18.6 %)	81.3 % (9.5 %)	QT prolongation	MONARCH-3 [110]
Abemaciclib	18.3	++	+	+	+	74.3 % (59.3 %)	32.9 % (21.0 %)	51.5 % (2.4 %)	16 (6 %)	36.5 % (2.4 %)	35.0 % (1.2 %)	Diarrhea, vein thrombosis, ILD/pneumonitis	MONALEESA-2 [111]

Abbreviations: CDK, cyclin dependent kinase; CNS, central nervous system; BC, breast cancer.

inhibitors. Notably, abemaciclib showed minimal activity against CDK2 as well [20]. Among the BC subtypes, HR + BC demonstrated the highest responsiveness to CDK4/6 inhibition, with the combination of CDK4/6i + ET becoming the standard of care for HR+/HER2- mBC.

Palbociclib was the first CDK4/6i to receive U. S. Food and Drug Administration (FDA) approval in combination with letrozole in treatment naïve metastatic patients following promising results from the phase II PALOMA-1 trial [21], subsequently confirmed by the phase III PALOMA-2 trial (progression free survival [PFS] 27.6 vs 14.5 months, hazard ratio [HR] 0.56, 95% confidence interval [95% CI] 0.46–0.69) [22]. The phase III PALOMA-3 trial evaluated fulvestrant and palbociclib in patients with disease progression on prior ET (PFS 9.5 vs 4.6 months, HR 0.46, 95% CI 0.36–0.59). Palbociclib showed a numerical but not statistically significant overall survival (OS) improvement only in the PALOMA-3 trial [23], while no clear OS benefit was observed in other clinical trials [21,22].

In the MONALEESA-2 trial, ribociclib and letrozole improved PFS and OS in the treatment-naïve setting (PFS 25.3 vs 16.0 months, HR 0.57, 95% CI 0.46–0.70; OS 63.9 vs 51.4 months, HR 0.76, 95% CI 0.63–0.93) [24]. The MONALEESA-3 trial demonstrated the efficacy of ribociclib plus fulvestrant compared to fulvestrant alone, showing a significant PFS and OS improvement (PFS 20.5 vs 12.8 months, HR 0.59, 95% CI 0.48–0.37; OS 53.7 vs 41.5 months, HR 0.73, 95% CI 0.59–0.90) [25]. Ribociclib is the sole CDK4/6i approved specifically for premenopausal patients based on the MONALEESA-7 trial, which included premenopausal patients receiving ovarian suppression regardless of AI backbone, demonstrating that the efficacy of ribociclib is comparable to that in postmenopausal cohorts (PFS: 23.8 vs 13 months, HR 0.55, 95% CI 0.44–0.69; OS: 58.7 vs 48 months, HR 0.76, 95% CI 0.61–0.96; OS: 58.7 vs 48 months, HR 0.76, 95% CI 0.61–0.96) [26].

Abemaciclib showed comparable efficacy in the first-line setting. In the phase III MONARCH-3 trial, the combination of abemaciclib with a non-steroidal AI (anastrozole or letrozole) improved PFS in first-line setting (28.2 vs 14.8 months, HR 0.54, 95% CI 0.42–0.70), with a numerical OS improvement (66.8 vs 53.7 months, HR 0.804, 95% CI 0.64–1.01) [27]. In the MONARCH-2 trial, fulvestrant plus abemaciclib significantly improved PFS and OS in patients with disease progression on prior ET (PFS: 16.4 vs 9.3 months, HR 0.55, 95% CI 0.45–0.68; OS: 45.8 months vs 37.2 months, HR 0.78, 95% CI 064–0.96) [28]. Abemaciclib is the only CDK4/6i approved by FDA as single agent in heavily pretreated patients, supported by the MONARCH-1 trial (overall response rate 19.7%, median PFS [mPFS] 6 months) [29].

The absence of head-to-head comparisons among CDK4/6i complicates the interpretation of discrepancies in terms of efficacy, leaving unresolved questions regarding the potential differences in overall survival outcomes among these agents. Real-world data from 9146 patients in the Flatiron U.S. database showed that the three approved CDK4/6i have similar OS in the first-line setting in HR+/HER2- mBC [30]. In the real-world PALMARES-2 study, both abemaciclib and ribociclib were associated with significantly improved real-world PFS compared with palbociclib, with adjusted hazard ratios of 0.76 (95% CI: 0.63–0.92; P = 0.004) and 0.83 (95% CI: 0.73–0.95; P = 0.007), respectively [31]. Further studies and translational analyses are needed to elucidate whether these observed variations reflect intrinsic differences between the inhibitors, patient population heterogeneity (such as the higher prevalence of Asian patients in PALOMA-2) or trial design nuances [32].

In contrast to palbociclib, abemaciclib and ribociclib have also demonstrated meaningful efficacy in the early setting [33,34]. The MonarchE trial demonstrated a significant benefit with two years of adjuvant abemaciclib. Among patients with high-risk features (≥4 positive lymph nodes or involvement of 1–3 lymph nodes and presence of T3 or G3 tumor characteristics), abemaciclib 150 mg BID per day plus ET improved invasive disease-free survival (iDFS) at two years (92.2 % vs. 88.7 %), with sustained benefit at four years (85.5% vs. 78.6%). In 2021, abemaciclib received FDA approval for adjuvant use in high-risk HR+/HER2- BC [9,10]. In the phase III NATALEE trial, ribociclib was

administered at a dose of 400 mg daily (21 days on/7 days off) for three years in patients with HR+/HER2- BC at high/moderate risk of relapse. The trial met its primary endpoint of iDFS benefit at three years (90.4% vs. 87.1%) [11]. In this study, the definition of a high-risk of recurrence was broader compared to MonarchE trial and also implemented genomic signatures such as OncotypeDX for patient selection.

The toxicity profiles of palbociclib, ribociclib, and abemaciclib differ significantly, providing opportunities for tailored treatment based on individual patient needs (Table 1). While cross-resistance between CDK4/6i is expected due to their similar mechanisms of action, clinical data suggest that switching between agents may be beneficial in cases of treatment-limiting toxicities [35]. New strategies involve the selective targeting of CDK4 or CDK6. Atirmociclib (PF-07220060) is a novel potent oral CDK4i with significant sparing of CDK6, which demonstrated a 24-week clinical benefit response in 20 (60.6 %) patients and a mPFS of 8.1 months (95% CI 5.3–10.9) in patients with HR+/HER2- BC pretreated with CDK4/6i [36].

Improving patients' outcomes: Novel combinations with CDK4/6 inhibitors

ET represents the natural companion for CDK4/6i in HR + BC since ER could stimulate proliferation through CDK4 level rise [4]. However, about 20 % of these tumors show intrinsic resistance with no response to treatment. In most of the other cases, initially responsive tumors will develop adaptive resistance mechanisms that drive progression [37]. Table 2 summarizes ongoing clinical trials exploring combinations of CDK4/6i and novel molecules, such as innovative ET (selective ER degraders [SERD], complete ER covalent antagonists [CERAN], targeted proteolysis chimera technology [PROTACs], selective ER covalent antagonists [SERCA]), *PIK3CA/AKT/PTEN* inhibitors, CDK2 inhibitors, and vascular endothelial growth factor (receptor) (VEGF[R]) inhibitors.

Improving ET backbone

CDK4/6i are administered in combination with ET. Selection of the optimal ET partner for CDK4/6i primarily depends on prior treatments received and disease-free interval. AIs are the preferred ET backbone unless disease progression occurs during or within 12 months from the end of adjuvant AI therapy, in which case, the SERD fulvestrant is recommended [6,38]. Notably, patient age or menopausal status does not influence this decision, as ovarian suppression in premenopausal patients ensures outcomes equivalent to those observed in postmenopausal populations. Therefore, tumor cells may develop resistance to ET, CDK4/6i, or both. *ESR1* mutation is the most characterized mechanism of acquired resistance to AI, described in up to 30 % of the cases [39,40]. SERDs are capable of targeting mutant ER, which would otherwise be able to promote gene transcription in a ligand-independent manner. Although fulvestrant demonstrated partial activity against mutant ER, it offers certain disadvantages in terms of administration (intramuscular injection), and exhibits less potent activity on both wild-type and mutant ER compared to novel oral SERDs [41]. Moreover, translational analysis from PALOMA-3 trial showed that *ESR1 Y537S* mutation is associated to resistance to fulvestrant and fulvestrant-palbociclib, further confirmed by preclinical data [42,43].

This detrimental effect of *ESR1* mutations appeared to be counterbalanced by new oral SERD monotherapy. In the EMERALD trial, elacestrant demonstrated a significant improvement in PFS compared to the standard-of-care ET, particularly in the *ESR1* mutated population and in the previously CDK4/6i sensitive population (i.e., prior ET + CDK4/6i $\geq$ 12 months) [44,45]. Elacestrant is an approved option in this setting; next-generation SERDs (e.g., camizestrant and imlunestrant) are in clinical development, and new adaptive trials investigating this strategy are currently underway [40].

Clinical data suggest that the combination of oral SERDs and CDK4/6i could represent a favorable option in HR+/HER2- mBC. In the phase I EMBER trial, the oral SERD imlunestrant was evaluated as a monotherapy and in combination with abemaciclib, with or without AI in CDK4/6i treatment-naïve patients. The mPFS was 19.2 months (95% CI 13.8-not available) for imlunestrant-abemaciclib and was not reached for imlunestrant-abemaciclib-AI. Antitumor activity was evident regardless of *ESR1* mutation status [46]. These preliminary data were further confirmed by the phase III EMBER-3, where patients progressing on AI with or without CDK4/6i were randomized 1:1:1 to receive imlunestrant, standard ET, or imlunestrant plus abemaciclib. The primary endpoint was PFS of imlunestrant vs standard therapy in patients with *ESR1* mutation and in overall population and PFS with imlunestrant-abemaciclib vs imlunestrant alone among all patients who had undergone randomization concurrently [47]. Treatment with imlunestrant resulted in significantly longer PFS than standard therapy among patients with *ESR1* mutations (5.5 vs 3.8 months; restricted mean survival time at 19.4 months, performed due to non-proportional hazards: difference, 2.6 months; 95% CI 1.2–3.9; $P < 0.001$) but not in the overall population (5.6 vs 5.5 months, HR 0.87; 95% CI 0.72–1.04). In contrast, imlunestrant–abemaciclib significantly improved PFS compared with imlunestrant, regardless of *ESR1*-mutation status (9.4 vs 5.5 months, HR 0.57; 95% CI 0.44–0.73; $P < 0.001$). The incidence of grade (G) $\geq$ 3 adverse events (AEs) was 17.1 % with imlunestrant, 20.7 % with standard therapy, and 48.6 % with imlunestrant–abemaciclib [47]. Although prior treatment with CDK4/6i was not mandatory for inclusion in the EMBER-3 trial, 65 % of the study population was pretreated with CDK4/6i. These results pose a challenge for the management of patients progressing to first-line or adjuvant CDK4/6i with *ESR1* mutation who are currently eligible for treatment with the oral SERD elacestrant, based on the EMERALD trial [44]. Additional trials are currently recruiting patients to assess the therapeutic efficacy and safety of different combinations of oral SERDs and CDK4/6i in both metastatic and early settings (Table 2).

Emerging evidence suggests that, beyond the mere presence of *ESR1* mutations, the dynamics of their development under therapeutic pressure may carry important biological and clinical implications. Indeed, following the initial treatment response, *ESR1* detection using liquid biopsy can be instrumental in the identification of resistance prior to radiological disease progression. In the PADA1 trial, patients were monitored using sequential liquid biopsies to detect emerging *ESR1* mutations during first-line palbociclib-AI. If an *ESR1* mutation was detected prior to radiological progression, patients were randomized to switch ET to fulvestrant or continue treatment with AIs. This strategy demonstrated a significant improvement in PFS, suggesting that monitoring the emergence of *ESR1* mutations may represent a strategy to optimize the ET backbone during treatment with a CDK4/6i [48]. Building upon evidence, the phase III SERENA-6 trial (NCT04964934) was designed. In this trial, upon detection of *ESR1* mutations in liquid biopsies during treatment, in the absence of clinico-radiologic progression, a transition from AIs to the next-generation oral SERD camizestrant while continuing CDK4/6i was evaluated [49].

Whether an early switch in ET backbone during first-line therapy with a CDK4/6i would be superior to using the alternative ET at disease progression has not been the objective of these trials. The concept of targeting a specific resistance mechanism at its earliest appearance may have profound implications for clinical practice to maximize the efficacy of CDK4/6i in the first-line setting. However, while the PADA-1 trial demonstrated clinical benefit from *ESR1* mutation-guided therapy switching, it's important to acknowledge that the correlation between mutation detection timing and clinical benefit is not perfect, and further refinement of biomarker-driven strategies is needed. Complete data from SERENA-6 will provide additional insights into this approach.

Future therapeutic combinations may involve the use of diverse endocrine agents, such as SERCA, CERAN, or PROTAC [41]. SERCAs inactivate ER, both in wild-type and mutant forms, by targeting a specific cysteine residue (C530). A phase I/II trial has tested the safety and preliminary efficacy of SERCA H3B-5942 with palbociclib in 10 patients,

Table 2
Ongoing trials investigating new combinations with CDK4/6 inhibitors in hormone receptor-positive, HER2-negative breast cancer.

	Trial ID	Phase	Mechanism of action	Cohorts	N	Primary Endpoint	Biomarker required	Setting
Endocrine therapy backbone	**NCT04964934 (SERENA-6)**	III	SERD	E: camizestrant + palbociclib/ abemaciclibC: anastrozole/letrozole + palbociclib/abemaciclib	300	PFS	*ESR1* mut	mBC
	NCT04711252 (SERENA-4)	**III**	SERD	E: camizestrant + palbociclibC: anastrazole + palbociclib	1342	PFS	–	mBC, ≥ 1L (AI or TAM pre-treated)
	NCT04546009 (persevERA)	**III**	SERD	E: giredestrant + letrozole-matching placebo + palbociclibC: letrozole + giredestrant-matching placebo + palbociclib	978	PFS	–	mBC, 1L
	NCT06065748 (pionERA)	**III**	SERD	E: giredestrant + abemaciclib/ palbociclib/ribociclibC: fulvestrant + abemaciclib/palbociclib/ribociclib	1050	PFS	*ESR1* mut	mBC, 1L
	NCT06062498	**II**	SERD	E: elacestrantE: elacestrant + palbociclib/ribociclib/abemaciclib	174	PFS	*ESR1* mut	mBC, ≥ 2L ET (CDK4/6i pre-treated)
	NCT05708235 (MiRaDoR)	**II**	SERD	E: giredestrant E: giredestrant + abemaciclib E: giredestrant + inavolisibC: control arm	1260	ctDNA clearance	–	Adjuvant; high risk
	NCT06259929 (Neo-AGILE)	**II**	SERD	E: giredestrant + abemaciclib	51	complete cell cycle arrest rate	–	neoadjuvant
	NCT05293964	**I**	SERD	E: SIM0270 E: SIM0270 + palbociclibE: SIM0270 + everolimus	214	MTD	–	mBC, without therapeutic options
	NCT05266105	**I**	CERAN	Palezestrant + palbociclib	30	DLT	–	mBC, Pretreated
	NCT05654623	**III**	PROTAC	E: vepdegestrant + palbociclibC: letrozole + palbociclib	1180	PFS	–	mBC, 1L
PIK3CA/AKT/ mTOR pathway	**NCT02389842 (PIPA)**	I	PI3KCA inhibitor	E: palbociclib + taselisib/pictilisib + fulvestrant	93	R2D	*PIK3CA* mut	mBC, ≥ 1L ET
	NCT02057133	I	PIK3CA/mTOR inhibitor	E: abemaciclib + letrozole/anastrozole E: abemaciclib + tamoxifene E: abemaciclib + exemestane E: abemaciclib + LY3023414 + fulvestrantE: Abemaciclib + everolimus + exemestane	123	n of patients with ≥ 1 AE	–	mBC, different requirements according the cohorts
	NCT05949541 (BCTOP-L-M01)	II	mTOR inhibitor	E: Dalpiciclib + everolimus + fulvestrant /letrozole/exemestaneC: dalpiciclib + fulvestrant /letrozole/ exemestane	265	PFS	SNF1 subtype	mBC, 1L
	NCT01872260 (X2107)	I	PI3K3CA inhibitor	E: Ribociclib + alpelisib + letrozole	300	DLT	–	mBC, 1L
	NCT05216432	I	PI3KCA inhibitor	E: palbociclib/ribociclib + RLY-2608 + fulvestrant	400	R2P	*PIK3CA* mut	mBC, ≥ 1L ET (CDK4/6i pre-treated); max 1L CT
	NCT03424005 (Morpheus-panBC)	I/II	PI3KCA inhibitor	E: Ribociclib + inavolisib + fulvestrant E: Ribociclib + inavolisib + letrozole E: abemaciclib + inavolisib + letrozoleE: abemaciclib + inavolisib + fulvestrant	580	ORR	–	mBC, pretreated
	NCT05768139	I/II	PI3KCA inhibitor	E: palbociclib/ribociclib + STX-478 + fulvestrant	400	DLT	*PIK3CA* mut	mBC, pretreated
	NCT05501886 (VIKTORIA-1)	III	PI3KCA/mTOR inhibitor	E: Palbociclib + gedatolisib + fulvestrant E: gedatolisib + fulvestrant C: fulvestrantC: alpelisib + fulvestrant	701	PFS	–	mBC, progression during or after CDK4/6i + AI
	NCT03959891 (TAKTIC)	I	AKT inhibitor	E: Palbociclib + ipasertib + fulvestrant	77	AEs	–	mBC, progression during or after CDK4/6i + AI
	NCT04862663 (CAPItello-292)	Ib/III	AKT inhibitor	E: palbociclib /ribociclib/abemaciclib + capivasertib + fulvestrant	895	DLT/PFS	–	mBC, eligible to CDK4/6 + fulvestrant
	NCT04920708 (FAIM)	II	AKT inhibitor	E: Palbociclib + ipatasertib + fulvestrant	324	PFS	–	mBC, eligible to CDK4/6 + fulvestrant
	NCT06561022 (abemaSNF1)	II	mTOR inhibitor	E: Abemaciclib + everolimus + fulvestrant	260	AEs	–	mBC, progression after CDK4/6i + AI
CDK2 inhibition	**NCT04553133**	I/II	CDK2 inhibitor	E: B monotherapy E: PF-07104091 + Fulvestrant + Palbociclib E: PF-07104091 + Letrozole + PalbociclibE: PF-07104091 + Fulvestrant	154	MTD	–	mBC, ≥ 2L, CDK4/6i pre-treated; max 2L CT
	NCT06188520 (CYCAD-1)	**I/II**	CDK2 inhibitor	E: AZD8421 monotherapy E: AZD8421 + Camizestrant + Abemaciclib E: AZD8421 +	204	DLT	–	mBC, pretreated

(*continued on next page*)

Table 2 (*continued*)

	Trial ID	Phase	Mechanism of action	Cohorts	N	Primary Endpoint	Biomarker required	Setting
	NCT05252416 (VELA)	**I/II**	CDK2 inhibitor	Camizestrant + RibociclibE: AZD8421 + Camizestrant + Palbociclib E: BLU-222 + Fulvestrant + Ribociclib E: BLU-22 + Carboplatin E: BLU-222 + Fulvestrant E: BLU-222 monotherapy	978	PFS	–	mBC, progression after CDK4/6i + AI
TME modulation	**NCT04355858 (MULAN)**	**II**	anti-PD-L1/TGF-βRII bifunctional fusion protein	E: Dalpiciclib + retlirafusp alfa (SHR-1701)	319	ORR	–	mBC, pretreated
	NCT06447623	**III**	VEGFR inhibitor	E: Apatinib + dalpiciclib + AI/ fulvestrantC: dalpiciclib + AI/ fulvestrant	184	PFS	SNF4 subtype	mBC, 1L
	NCT05759572	**III**	VEGFR inhibitor	E: apatinib + dalpiciclib + fulvestrant/ AI E: dalpiciclib + fulvestrant/AI	145	PFS	SNF4 subtype	mBC, 1L
	NCT06307249 (PTST_PALBEVA)	**I**	Anti-VEGF mAb	Palbociclib + bevacizumab	50	PD	–	mBC, pretreated

Abbreviations: mut, mutation; mBC, metastatic breast cancer; 1L, first-line; 2L, second-line; ET, endocrine therapy; N, planned sample size; MTD, maximum tolerated dose; DLT, dose limiting toxicity; BC, breast cancer; SCLC, small cell lung cancer; N, planned sample size; PFS: progression free survival; AE: Adverse events, ORR, overall response rate; PD, progressive disease; mAb, monoclonal antibody; AI, aromatase inhibitors; TAM, tamoxifen; CDK4/6i, CDK4/6 inhibitors; L, line; CT, chemotherapy; TME, tumor microenvironment. Notes: cohorts enrolling other BC IHC subtypes or other tumors are excluded from the table.

reporting G3/4 neutropenia and thrombocytopenia, G3 hypercalcemia, G3 lipase increase and G1/G2 bradycardia as treatment related AEs [50].

CERANs antagonize the transcriptional activity of ER, even in the presence of estrogen. Palazestrant (OP-1250) is a CERAN and a SERD that completely blocks ER-driven transcriptional activity. Preliminary results from an ongoing phase Ib/II study (NCT05508906) showed a manageable safety profile of palazestrant in combination with ribociclib in 27 patients with HR+/HER2– mBC with ≤ 2 prior ET and ≤ 1 prior line of chemotherapy [51]. Additionally, the combination of palezestrant and palbociclib demonstrated a good safety profile and no drug–to-drug interactions in a phase Ib/II trial (NCT05266105) enrolling patients with HR+/HER2- BC treated with ≤ 1 prior ET and ≤ 1 prior line of chemotherapy [52].

PROTACs induce ubiquitination of target proteins and their subsequent degradation by the ubiquitin–proteasome system [40]. ARV-471 (vepdegestrant) is an ER PROTAC that targets ER alpha and forms a heterobifunctional PROTAC-degrading wild-type and mutant ER. According to the phase I/II trial VERITAC (NCT04072952), ARV-471 showed evidence of clinical activity and a manageable safety profile (200 mg QD: clinical benefit rate [CBR] 37.1 %, 95% CI 21–55; 500 mg QD: CBR 38.9 %, 95% CI 23–57) in heavily pretreated patients HR+/HER2 − mBC, which was further enhanced in the *ESR1* mutant subgroup (200 mg QD: CBR 47.4 %, 95% CI 24–71; 500 mg QD: CBR 54.5 %, 95% CI 32–76) [53]. The phase III trial VERITAC-2 (NCT05654623) is currently ongoing to assess the efficacy of vepdegestrant compared to fulvestrant after CDK4/6i, while the phase III VERITAC-3 (NCT05909397) is testing vepdegestrant-palbociclib vs. palbociclib-letrozole in first-line setting.

All the ongoing trials aiming to improve ET backbone are summarized in Table 2**.**

Targeting genomic resistance mechanisms to CDK4/6 inhibitors

- *PIK3/AKT/mTOR pathway*

Dysregulation of the PIK3/AKT/mTOR signaling pathway has been observed in both BCs with de novo and acquired resistance to ET and CDK4/6i [42,54–56]. In the PALOMA-3 trial, *PIK3CA* mutation emerged as an acquired mutation following treatment with palbociclib-fulvestrant (P = 0.00069). In preclinical models, concomitant inhibition of ER, CDK4/6 and PIK3 pathways elicits synergistic effects on cell cycle arrest, apoptosis and cell death, overcoming intrinsic and adaptive resistance [57–59]. In this setting, alpelisib (a selective PI3KCA inhibitor) and capivasertib in combination with ET are options showing benefit in clinical setting.

Focusing specifically on CDK4/6i resistance, the phase I/II TRINITI-1 trial (NCT02732119) demonstrated that mTOR inhibitors can potentially restore sensitivity to ET, with a CBR at 24 weeks of 41.1 % of combination of ribociclib, everolimus, and exemestane in patients progressing after at least 4 months of CDK4/6i therapy [60]. A comparable study design was employed in a separate phase Ib/IIa trial (NCT02871791), which evaluated the safety and tolerability of the combination of exemestane, everolimus and palbociclib in 32 patients with HR+/HER2- mBC after CDK4/6i failure. The primary objective of CBR was not reached (18.8 %); however, multi-omics analyses conducted on baseline samples (including *ESR1* mutations, high ER pathway activity, and a Luminal A/B subtype; *ERBB2/BRAF* mutations, high receptor-tyrosine kinase/MAPK pathway activity, and a HER2-enriched subtype) suggested that mTOR pathway activation may predict the response to triplet therapy [61]. The extent to which the differences between the two trials could be attributed to distinct study populations or the different CDK4/6i used remains a subject of ongoing debate.

The combination of *PIK3CA* inhibitors and CDK4/6i demonstrated enhanced efficacy in suppressing RB phosphorylation [59]. Inavolisib is a potent and selective p110α inhibitor that promotes the degradation of mutated p110α. In the phase III INAVO-120 trial (NCT04191499), the combination of inavolisib plus palbociclib–fulvestrant resulted in significantly longer PFS compared to placebo plus palbociclib–fulvestrant (15.0 vs 7.3 months, HR 0.43, 95% CI 0.32–0.59) in patients with *PIK3CA* mutated, HR+/HER2- mBC progressing within 12 months of completion of adjuvant treatment [62]. However, the triplet combination was less tolerated than the standard therapy, with a higher incidence of G3/4 neutropenia (80.2 % vs. 78.4 %), hyperglycemia (5.6 % vs. 0 %), despite strict inclusion criteria (HbA1c < 5.7 %), stomatitis or mucosal inflammation (5.6 % vs. 0 %), and diarrhea (3.7 % vs. 0 %), resulting in a higher discontinuation rate (6.8 % vs. 0.6 %). Based on the INAVO-120 trial, the FDA recently approved the triplet combination in the first-line setting following recurrence on or after adjuvant ET. Innovative pharmacological designs involve dual targeting of *PIK3CA* pathway, as for gedatolisib – an ATP-competitive and reversible dual PI3K/mTOR inhibitor [63], currently under evaluation with fulvestrant +/- palbociclib in the phase III VIKTORIA-1 trial (NCT05501886) for HR+/HER2- mBC after CDK4/6i progression.

In preclinical models, concomitant CDK4/6 and AKT inhibition efficiently blocks cyclin D/CDK4-6/Rb and PI3K/AKT-mTOR pathways in HR + BC cell lines, demonstrating superior efficacy compared to the

single agent therapies [64]. Additionally safety data also come from preliminary results of the phase Ib/III study CAPitello-292, evaluating the safety and efficacy of the AKT inhibitor capivasertib with palbociclib-fulvestrant in HR+/HER2- mBC. Preliminary data from phase Ib part of the study have been recently presented: in 39 heavily pretreated patients, most common AEs were diarrhea (69 %; 1/27 G3), neutropenia (54 %; 19/21 G $\geq$ 3), fatigue, and nausea (41 %; all G1/2) [65]. The relative efficacy of capivasertib or inavolisib as CDK4/6i partner remains undetermined. Consideration should be given to the safety profile of the two different drugs as well as the toxicity of the candidate CDK4/6i. To date, several studies have exploited palbociclib, which appears to exhibit lower toxicity and fewer drug-to-drug interactions but potentially reduced efficacy compared to the other two approved CDK4/6i [66]. Moreover, the genomic determinants of response/resistance to treatment should be considered. In particular, the presence of alterations such as *PTEN* loss could potentially lead to cross resistance to both CDK4/6i and *PIK3CA* inhibitors [67].

- *FGFR pathway*

In preclinical models, *FGFR1* overexpression has been shown to induce resistance to CDK4/6i alone and in combination with ET, a phenomenon that can be restored through FGFR inhibition [68]. Additionally, the presence of *FGFR1* alterations was associated with shorter PFS in the MONALEESA-2 trial [68]. However, in MONALEESA-3 and MONALEESA-,2 the benefit of ribociclib was observed irrespective of baseline ctDNA *FGFR1* alterations [69]. Although prior attempts to combine FGFR inhibitors with ET in HR + mBC did not change clinical practice [70], the combination of FGFR inhibitors with CDK4/6i and ET is being actively investigated. In patients with *FGFR* pathway amplification, a phase II trial (NCT01528345) explored the combination of fulvestrant with either dovitinib, a potent multi-target kinase inhibitor (including FGFR1 and FGFR3), or placebo showing an improved PFS in the experimental arm (mPFS 10.9 vs 5.5 months) [71]. The combination of palbociclib, fulvestrant, and the pan-FGFR inhibitor erdafitinib is currently under investigation in a phase Ib trial in patients with HR+/HER2-/*FGFR1-4* amplified mBC (NCT03238196). However, the safety profile of this combination was not satisfying, leading to treatment discontinuation in several patients; moreover, survival outcomes were not excellent (mPFS 3 months; CBR at 6 months 28 %) [72].

- *Focus on other CDKs*

The CDK2/Cyclin E complexes play a major role in the transition to the S-phase by phosphorylating Rb1 in a positive feedback loop, thereby fully activating E2F. Cyclin E is encoded by *CCNE1* gene. Interestingly, biomarker analysis from the elevated PALOMA-3 trial demonstrated that high levels of *CCNE1* mRNA correlate with a diminished response to palbociclib [42]. Preclinical evidence suggests that Cyclin E overexpression leads to resistance to ET and CDK4 inhibition but not to CDK2 inhibition [73]. Therefore, the inhibition of CDK2 might be a potential strategy to overcome cell plasticity in tumors resistant to ET and CDK4/6i, as shown by preclinical cell lines models exploring the effect of pyrimidine derivatives [74]. Early phase studies (NCT04553133, NCT05252416, NCT05735080, NCT06726148) are investigating novel CDK2 inhibitors (PF-07104091, AZD8424, INX-315-01, ECI830) in combination with ET +/- CDK4/6i. BLU-222 is a selective CDK2 inhibitor in clinical development. In the phase 1/2 VELA study (NCT05252416), 64 patients with advanced solid tumors, including heavily pretreated HR+/HER2-mBC, received BLU-222 as a monotherapy or in combination with ribociclib-fulvestrant. The treatment was well-tolerated, with gastrointestinal symptoms, fatigue, and photophobia as common AEs. In the combination cohort, initial data showed that BLU-222, when added to ribociclib-fulvestrant, did not present additional safety concerns compared to the individual therapies [75]. PF-07104091, a novel CDK2-selective inhibitor, was evaluated in a first-in-human phase 1 study in patients with advanced solid tumors, including heavily pretreated HR+/HER2- mBC. Among the 35 enrolled patients (29 BC), treatment was generally well-tolerated, with common AEs including nausea, fatigue, and anemia. Preliminary efficacy showed a disease control rate of 61.5 % in response-evaluable mBCs, with partial responses in 18.8 % [76]. The CYCAD-1 trial (NCT06188520) is exploring the efficacy of the CDK2 inhibitor AZD8421 in monotherapy or in combination with the oral SERD camizestrant and CDK4/6i.

Preclinical studies suggest that co-inhibition of CDK2 and CDK4/6 may be necessary to overcome intrinsic cell cycle plasticity [77]. An ongoing multicenter dose-escalation phase I/IIB trial (NCT05262400) is currently evaluating the combination of CDK2 and CDK4 inhibition in advanced solid tumors, including BC. In this study, atirmociclib (PF-07220060, a CDK4 inhibitor) was combined with PF-07104091 (a CDK2 inhibitor) in patients with HR+/HER2– mBC and other advanced solid tumors. Among 33 patients, the combination was well-tolerated. In the mBC subgroup, 27.8 % of the evaluable patients achieved partial responses (all harboring *ESR1* mutations), with an mPFS of 8.3 months and a disease control rate of 69.2 % [78].

Preclinical studies have demonstrated the sensitivity of many cancers to novel selective CDK7 inhibitors and CDK4 inhibitors [79]. Recently, samuraciclib, an oral CDK-7 inhibitor, has demonstrated clinical activity in a phase I clinical trial, particularly in luminal mBC patients without *TP53* mutations, with a CBR of 47.4 % [80].

- *DNA repair mechanism*

Retrospective analyses from MONALEESA trials suggest that patients with g*BRCA* mutations may derive less benefit from CDK4/6i compared to those with wild-type *BRCA* [69]. These data were supported also by real world evidence [81]. *BRCA1* is crucial for the p21-mediated G1/S transition [82], a key target of CDK4/6i, and its loss may disrupt the intended mechanism of action of these agents. The concurrent deletion of *BRCA2* and *RB1*, located proximally on chromosome 13q, may influence therapeutic responses [83]. Furthermore, *BRCA1*-deficient tumors often exhibit hyperactivation of the *PI3K/AKT* pathway [84,85], already discussed as a well-known driver of ET and CDK4/6i resistance. To address these limitations, novel therapeutic strategies are being explored. The HOPE trial (NCT03685331) attempted to combine the PARP inhibitor olaparib with palbociclib-fulvestrant in *gBRCA*-mutated HR+/HER2- mBC; however, excessive myelotoxicity led to early termination [86]. An ongoing phase III trial (NCT06612814) is comparing the combination of dalpiciclib, fluzoparib and ET vs. dalpiciclib plus ET in patients with HR+/HER2- BC.

Shaping epigenetics

KAT6A gene encodes for an histone acetyltransferase and is amplified in 12–15 % of BCs [87]. PF-07248144, a potent and selective catalytic KAT6 inhibitor, is currently undergoing evaluation in patients with solid tumors in a phase I trial (NCT04606446). Data from the monotherapy and the fulvestrant combination arms were promising in terms of toxicity (G3/4 neutropenia 35.5 %, G3/4 anemia 13.1 %), with the most common AEs being dysgeusia (all G 83.2 %), and in terms of activity (ORR 30.2 % [95% CI 17.2–46.1], mPFS 10.7 months [95% CI 5.3–not evaluable]) [88]. Results from the arm evaluating PF-07248144 in combination with letrozole-palbociclib are awaited [88]. Other first-in-human KAT6 inhibitors under investigation are MEN2312 (NCT06784193, in patients with mBC progressing to CDK4/6i), and OP-3136 (NCT06784193, in solid tumors including BC).

Modeling the tumor microenvironment

The use of CDK4/6i in combination with immune checkpoint inhibitors (ICIs) has generated considerable interest due to the potential synergistic antitumor immune responses. Indeed, preclinical evidences

have shown that CDK4/6i could trigger anti-tumor immunity through various mechanisms: CDK4/6i might enhance MHC-I antigen presentation, suppress regulatory T cells, and ultimately activate CD8 + T cell [89,90]. Moreover, activation of the interferon signaling pathway is also associated with resistance to CDK4/6i [91] and PD-L1 protein expression is regulated by Cyclin D/CDK4 [92]. In preclinical models, CDK4/6 inhibition improve efficacy of ICI, enhancing expression of immune related signatures [89,92]. However, multiple clinical trials have revealed significant challenges, particularly in terms of toxicity and limited efficacy. Pembrolizumab was tested in combination with palbociclib (with letrozole) or abemaciclib (with or without anastrozole) in two early phase clinical trials (NCT02778685 and NCT02779751, respectively). Although the promising disease control rate (23.1 %), abemaciclib-pembrolizumab showed higher rates of interstitial lung disease/pneumonitis compared to previously reported rates with abemaciclib and pembrolizumab monotherapy [93]. The combination of palbociclib, letrozole and pembrolizumab was also not excellent in terms of safety, with 30 % of the patients experiencing a grade 4 AEs [94]. Concerns regarding the safety of ICIs-CDKs combinations were further confirmed by the combination of the anti-PD-1 spartalizumab with ribociclib, which resulted in high rate of hepatotoxicity (ALT elevation G $\geq$ 2 66.7 %) and limited clinical efficacy (CBR 26.7 %) in patients with HR+/HER2- mBC [95]. The PD-L1 inhibitor atezolizumab was tested in in the second- or third-line setting post-CDK4/6i in MORPHEUS HR + trial. In this study, the combination of atezolizumab-abemaciclib-fulvestrant showed an improved ORR and PFS compared with fulvestrant monotherapy. However, treatment related AEs led to discontinuation in approximately 20.8 % of the patients in the triplet arm [96]. In the phase II PACE trial, patients with HR+/HER2- BC progressing on CDK4/6i-AI were randomized to receive fulvestrant, fulvestrant-palbociclib or triplet fulvestrant, palbociclib, and avelumab. Although the primary endpoint of PFS in patients treated with fulvestrant vs fulvestrant-palbociclib was not met (4.8 vs 4.6 months, HR 1.11, 90 % CI 0.79–1.55), a numerically encouraging trend was seen in the triplet arm (8.1 months) [97]. Other strategies targeting the tumor microenvironment (TME) in combination with CDK4/6i include the use of anti-PD-L1/TGF-βRII bifunctional fusion protein (retlirafusp alfa or SHR-1701, NCT043558588/MULAN trial), VEGF(R) inhibitors such as apatinib (NCT06447623, NCT05759572) or bevacizumab (NCT06307249), or the modulation of prostaglandin (NCT06570031).

In conclusion, while CDK4/6i show promise in enhancing antitumor immunity and sensitizing HR+/HER2 − BC to ICIs, clinical translation has been hindered by significant toxicities and limited efficacy in several combination regimens. Future success will depend on the development of biomarker-driven strategies, improved patient selection, and rational combination approaches that safely and effectively modulate the TME.

Adding efficacy, sparing toxicities: The balance of triplet therapy

Combining CDK4/6i with other therapeutic agents may be highly effective in treating HR+/HER2- BC. However, it also presents challenges including increased toxicity, cost, and patient adherence. In particular, toxicity can limit the safety of long-term treatments. One major concern is the risk of neutropenia and leukopenia, which are AEs of CDK4/6i and may be challenging when considering targeted therapies such as other CDK inhibitors. To mitigate this risk, careful patient selection will be crucial, along with intensive laboratory monitoring, with immediate dose adjustment or treatment interruption. Gastrointestinal toxicity, including diarrhea and nausea, is particularly associated with abemaciclib. These AEs can significantly affect patients' quality of life, and when combined with other therapies having possible gastrointestinal disturbances (i.e., immunotherapy and *PIK3CA* inhibitors), the daily burden on the patient can be substantial, potentially leading to noncompliance. Supportive care measures should include closely monitor symptoms and provide proactive management with antiemetic and antidiarrheal medications, along with dietary adjustments. Liver toxicity is another concern, particularly with ribociclib or immunotherapy. Liver function tests should be monitored regularly with subsequent dose adjustments or discontinuation of different drugs. Considering less hepatotoxic alternatives, with careful management of drug-drug interactions, may help to reduce this risk.

Clinicians should consider the burden of concurrent therapy, especially for elderly patients, given the potential risk of drug-drug interactions. Indeed, many targeted therapies are metabolized by the liver enzyme CYP3A4, and drugs that modulating its activity can affect the levels of CDK4/6i, with subsequent increased toxicity or reduced efficacy. Careful review of all concomitant medications is essential to avoid significant interactions. Dose adjustments or alternative therapies may be necessary to maintain the safety and effectiveness of treatment regimens.

Finally, financial toxicity will be a critical challenge. The approval and integration of novel therapies in combination with CDK4/6i will substantially increase treatment costs, particularly in post-CDK4/6i setting. This escalation may restrict global access to these innovative therapies, further exacerbating disparities in the management of mBC.

Future perspectives: Treatment algorithms and triplets

As described above, several clinical trials are currently investigating triplet therapy strategies across various clinical settings. Two primary areas of focus have emerged: CDK4/6i naïve patients and those who progress after CDK4/6i treatment. At present, molecular profiling of HR+/HER2- tumors via solid or blood circulating tumor DNA (ctDNA) is indicated upon progression to CDK4/6i, with the objective of identifying actionable alterations such as *PIK3CA*, *AKT*, *BRCA1/2*, *PTEN*, *ESR1* mutations [38,98,99]. Specifically, the identification of a *PIK3CA* hotspot mutation serves as an eligibility criterion for treatment with alpelisib and ET, based on the results of the SOLAR-1 and BYLIEVE studies [100–102]. Similarly, alteration of *PI3K/PTEN/AKT* pathway indicated a possible benefit from treatment with the *AKT* inhibitor capivasertib, according to the CAPITELLO-291 trial [103]. The presence of a g*BRCA1/2* mutation confers eligibility for olaparib or talazoparib treatment. Presence of an *ESR1* mutation serves as an eligibility criterion for elacestrant use in the advanced setting based on the EMERALD trial [44]. However, these indications may be subject to modifications in the future as results from ongoing clinical trials become available. For example, data from the phase III INAVO-120 trial (NCT04191499) led to FDA approval for front-line combination of inavolisib plus palbociclib–fulvestrant in patients with *PIK3CA* mutated, HR+/HER2- mBC progressing within 12 months of completion of adjuvant treatment [104]. To select these patients, molecular profiling will be required, even in the absence of prior CDK4/6i treatment. Escalation with triplet therapy may be advised in patients relapsing after one year from completion of adjuvant CDK4/6 inhibitors, who have not shown a primary resistance to both ET and CDK4/6i.

Other strategy involve the use of CDK4/6i beyond progression. Based on data from the PostMONARCH and MAINTAIN trials, patients with prior CDK4/6i treatment lasting $\geq$ 12 months showed better PFS when continuing with CDK4/6i [105,106]. In PostMONARCH trial, abemaciclib plus fulvestrant provided meaningful benefit compared to standard therapy after progression to CDK4/6i, particularly in those who benefited the most from prior CDK4/6i. The identification of predictive biomarkers of response/resistance through molecular profiling remains critical for optimizing patient selection for triplet therapies. Identifying tumor characteristics that could predict sensitivity or resistance to CDK4/6i and ET would facilitate the optimization of strategies, directing patients to innovative clinical trials aiming at maximizing clinical benefits while minimizing toxicities.

Liquid biopsy profiling will be useful also in identifying early dynamic changes in ctDNA that could serve as biomarkers for identifying patients who may require additional therapeutic interventions. Following the start of CDK4/6i, patients who respond to treatment

typically exhibit a decrease in ctDNA levels, whereas non-responders generally maintain ctDNA levels comparable to baseline [107]. The phase IIIb BioItaLEE trial (NCT03439046) demonstrated that both pre-treatment and dynamic assessment of ctDNA have prognostic and predictive value in patients with HR+/HER2- mBC treated with first-line ribociclib and letrozole [108]. The phase II FAIM trial (NCT04920708), which is currently recruiting participants, focuses on patients whose ctDNA levels remain unchanged relative to baseline after two weeks of CDK4/6i treatment in the metastatic setting. The trial randomizes either to add the *AKT* inhibitor ipatasertib to the ongoing treatment or to continue the same therapy.

Translational analysis from both ongoing and completed trials will be essential for refining these biomarker-driven approaches. Fig. 2 illustrates how the treatment algorithm may evolve soon, informed by available results of recently published or presented trials.

Conclusions

Preclinical and translational research has elucidated multiple strategies to extend and enhance the therapeutic efficacy of CDK4/6i beyond their standard use with AI or fulvestrant. Potential candidates encompass a variety of molecules, including novel ET, ICIs, and targeted therapies inhibiting CDK2, *PIK3CA/AKT1/PTEN*, or other pathways.

Among these emerging strategies, several approaches stand out as particularly promising. The combination of CDK4/6i with PIK3CA/AKT pathway inhibitors represents one of the most advanced strategies, with compelling clinical data from the INAVO-120 trial establishing inavolisib plus palbociclib-fulvestrant as an effective option for *PIK3CA*-mutated HR+/HER2- BC [104]. The significant improvement in PFS (15.0 vs 7.3 months, HR 0.43) underscores the potential of targeting this frequently altered pathway [104]. Similarly, capivasertib has shown promise in the CAPItello-291 trial, suggesting that AKT inhibition may be a viable strategy for a broader patient population, including those without specific PIK3CA mutations [65].

Novel endocrine therapy approaches also merit particular attention. Next-generation SERDs and other ER-targeting agents (CERAs, PROTACs) could address endocrine resistance mechanisms, especially ESR1 mutations. The positive results of the SERENA-6 trial highlight the potential value of early intervention upon detection of emerging *ESR1* mutations, while data from the EMBER-3 trial suggest the potential efficacy of combining oral SERDs with CDK4/6i in patients progressing on prior therapy [46]. These approaches represent a paradigm shift toward adaptive, biomarker-guided therapeutic strategies.

The dual targeting of cell cycle control points through combined CDK4/6 and CDK2 inhibition is another highly promising avenue. Preclinical and early clinical data suggest that this approach may overcome the plasticity in cell cycle regulation that contributes to CDK4/6i resistance. Recent data from the combination of CDK4-specific inhibitor PF-07220060 and CDK2 inhibitor PF-07104091 have shown encouraging clinical activity, particularly in *ESR1*-mutated tumors, warranting further investigation in larger trials [36,76,78].

Future research priorities should focus on several key areas:

1. Biomarker development and validation: There is an urgent need to identify and validate predictive biomarkers beyond *PIK3CA* and *ESR1* mutations to better select patients for specific combination therapies. Dynamic assessment of treatment response through liquid biopsies should be incorporated into standard clinical practice and trial designs.
2. Optimal sequencing strategies: Determining the ideal sequence of therapies to maximize long-term outcomes remains a critical question. Future trials should evaluate whether certain combinations are most effective in specific treatment lines or whether concurrent use of multiple targeted agents from the outset might prevent resistance development.
3. De-escalation strategies: Given the potential toxicities of combining multiple targeted agents, research should also focus on identifying patients who might benefit from less intensive approaches, including

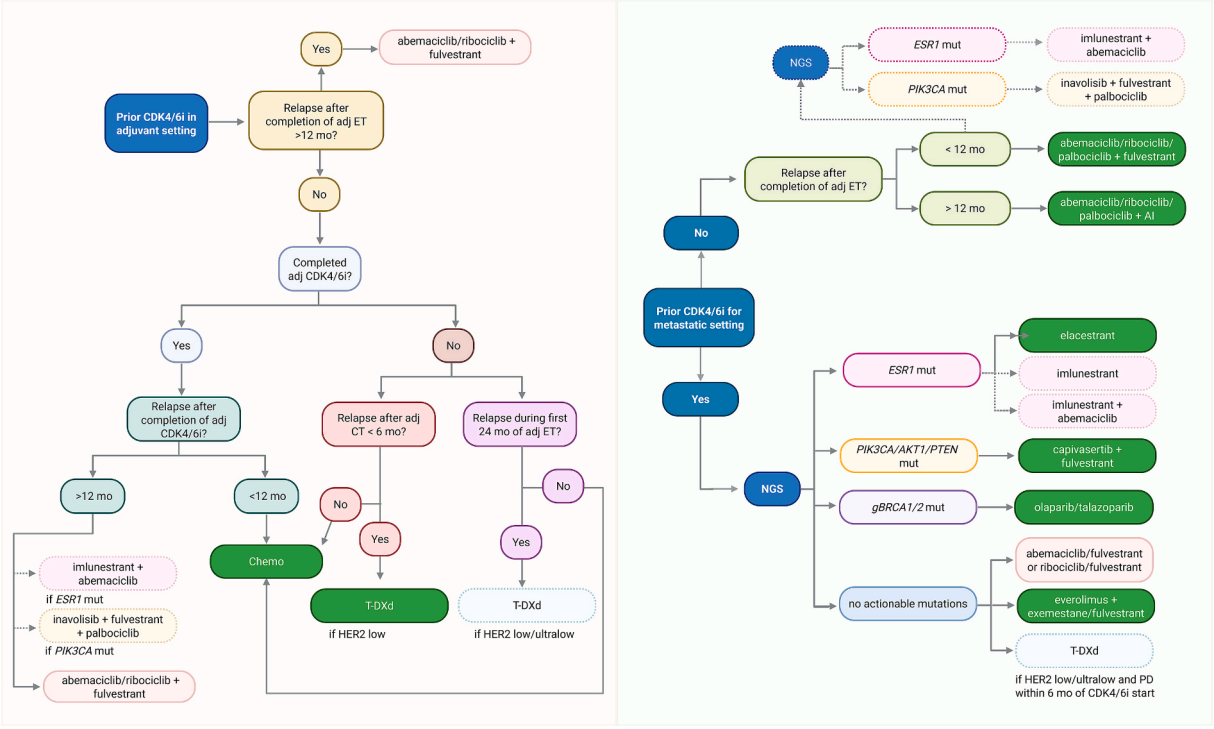

Fig. 2. Potential treatment algorithm for hormone receptor-positive, HER2-negative breast cancer based on published data from prospective clinical trials or data presented at international congresses. In green, options approved by EMA. Abbreviations: NGS, next-generation sequencing; PD, progressive disease; CDK4/6i, CDK4/6 inhibitor; ET, endocrine therapy; AI, aromatase inhibitors; T-DXd, trastuzumab deruxtecan; adj, adjuvant; chemo, chemotherapy. Created using biorender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

intermittent dosing schedules or strategic drug holidays to improve tolerability without compromising efficacy.
4. Novel combinations targeting the TME: While initial attempts to combine CDK4/6i with ICIs have shown mixed results, further exploration of approaches to enhance immune recognition of HR+/HER2- BC, traditionally considered "immunologically cold," could yield significant advances.
5. Mechanisms of acquired resistance: Deeper understanding of the molecular mechanisms of acquired resistance to combination therapies, potentially through analysis of paired biopsies before and after progression, could inform the development of new therapeutic strategies.
6. Leveraging real-world data: Integrating real-world evidence into research efforts is essential, particularly for understanding treatment effectiveness and tolerability in patient populations often underrepresented (elderly patients, patients with comorbidities, patients living in Countries with limited access to liquid biopsy or molecular profiling…) in clinical trials. The use of high-quality real world data can complement clinical trial findings, guide clinical decision-making, and help refine patient selection for future studies.

International collaboration will play a pivotal role in accelerating the clinical translation of these findings. Coordinated efforts across research institutions, clinical trial networks, and regulatory bodies will be essential to overcome challenges, harmonize methodologies, and facilitate the efficient introduction of innovative treatments to patients. Moreover, addressing the financial toxicity associated with these novel combinations through innovative pricing models and cost-effectiveness analyses will be crucial to ensure equitable access to these promising therapeutic strategies globally.

As we enter this new era of precision oncology in HR+/HER2- BC, the integration of molecular profiling, adaptive trial designs, and real-world evidence will be instrumental in optimizing treatment approaches and improving long-term outcomes for patients with this common breast cancer subtype.

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CRediT authorship contribution statement

Federica Giugliano: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Carmine De Angelis:** Writing – original draft, Writing – review & editing. **Barbara Pistilli:** Writing – review & editing. **Giulia Viale:** Writing – review & editing, Writing – review & editing. **Giampaolo Bianchini:** Writing – review & editing. **Mario Giuliano:** Writing – review & editing. **Luca Malorni:** Writing – review & editing. **Beatrice Taurelli Salimbeni:** Writing – review & editing. **Angela Esposito:** Writing – review & editing. **Antonio Giordano:** Writing – review & editing. **Timothy A. Yap:** Writing – review & editing. **Giuseppe Curigliano:** Writing – review & editing. **Carmen Criscitiello:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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