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Therapeutic Use of CDK4/6 Inhibitors in Hormone Receptor–Positive, HER2-Negative Advanced Breast Cancer: Underlying Molecular Mechanisms, Clinical Impact, and Prospective Directions

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ABSTRACT

Hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer represents the most prevalent subtype, with endocrine therapy (ET) serving as the primary treatment approach. While anti-estrogen therapies are initially effective in most patients, around 50% of HR+ cases eventually develop resistance to ET, resulting in disease recurrence and limited long-term benefit. The introduction of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors—palbociclib, ribociclib, and abemaciclib—combined with ET has significantly improved outcomes in HR+ advanced breast cancer (ABC) by targeting cell-cycle regulation and mitigating certain mechanisms of endocrine resistance. Nonetheless, identifying which patients are most likely to benefit, defining the key characteristics for optimal patient selection, and discovering predictive biomarkers of response remain unresolved. This review discusses the mechanisms of action of CDK4/6 inhibitors, potential resistance pathways, their clinical implications, and emerging strategies aimed at enhancing their effectiveness to improve survival and quality of life in patients with HR+, HER2− ABC.

Keywords: Endocrine resistance, Breast cancer, CDK4/6 inhibitors, Advanced breast cancer (ABC), Endocrine therapy (ET)

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Introduction

The most frequently diagnosed subtype of breast cancer is hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, with endocrine therapy (ET) remaining the primary treatment strategy. Although patients often respond well to anti-estrogen therapies initially, around 50% eventually experience resistance, resulting in disease recurrence and reduced therapeutic benefit [1]. The addition of cyclin-dependent kinase 4 (CDK4) and CDK6 inhibitors—palbociclib, ribociclib, and abemaciclib—to ET has substantially improved outcomes in HR+ advanced breast cancer (ABC) by interfering with cell-cycle regulation and partially counteracting mechanisms of endocrine resistance.

Pathways driving endocrine therapy resistance

Resistance to ET in breast cancer has been largely attributed to three regulatory mechanisms affecting estrogen receptor (ER) gene (ESR1) expression [2]: (i) Classic signaling: mutations in the ER ligand-binding domain that trigger ESR1 activation, seen in roughly 18% of resistant HR+ cases; (ii) Ligand-independent signaling: ER activation induced by downstream signals from receptor tyrosine kinases (RTKs); and (iii) Non-genomic signaling: ER-mediated pathways occurring at the plasma membrane or within the cytoplasm of tumor cells. A visual summary of these resistance mechanisms is provided in **Figure 1**.

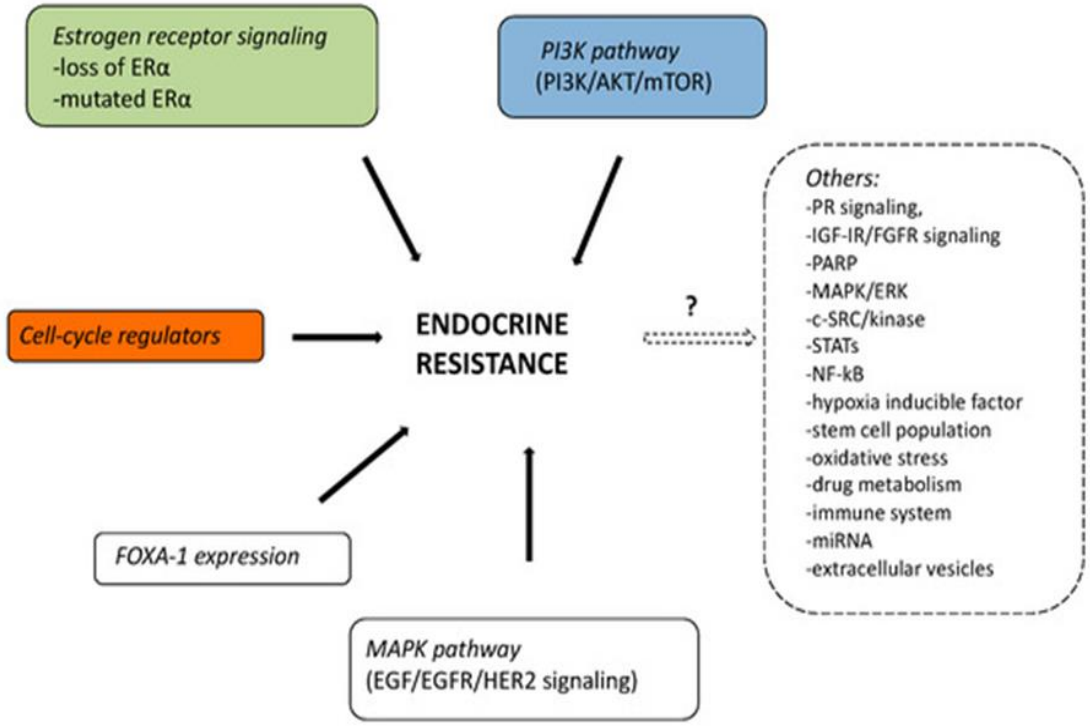

Figure 1. Possible mechanisms of endocrine resistance in summary.

ER-α mutations

Mutations in ER are uncommon in primary breast tumors but tend to appear more frequently as tumors acquire resistance to endocrine therapy [3]. These point mutations promote estrogen-independent transcriptional activity, driving cancer cell proliferation and contributing to endocrine therapy resistance [4].

ER-α Loss

The absence of ER is a major factor in de novo resistance to ET. Downregulation of ER-α can occur via epigenetic alterations, including CpG island methylation or histone deacetylation within the ESR1 promoter; DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) modulate chromatin structure, thereby controlling ER gene expression [5]. Laboratory studies have shown that DNMT1 inhibitors (e.g., Aza) and HDAC inhibitors (e.g., TSA) reduce chromatin compaction, restoring ER expression in ER-negative breast cancer cell lines [6]. In animal models, combined treatment with AZA and TSA inhibited tumor growth following ovarian ablation and reinstated tamoxifen sensitivity in ER-negative tumors [7].

MAPK pathway (EGF/EGFR/HER2 Signaling)

Alterations in the MAPK signaling cascade are reported in roughly 13% of breast cancers [1]. Beyond common ESR1 hotspot mutations, ERBB2 and NF1 mutations—typically mutually exclusive—show the greatest variation between pre- and post-endocrine therapy in HR+ HER2 tumors [1]. Tamoxifen-resistant breast cancer cells (LTam) exhibit hyperactivation of the HER/EGFR/Akt/ERK pathway, and preclinical studies demonstrate that lapatinib, a dual EGFR/HER2 inhibitor, can restore tamoxifen responsiveness in these resistant cells [8].

PI3K pathway (PI3K/AKT/mTOR)

Resistance to aromatase inhibitors, such as letrozole, is linked to heightened activity of p70S6K and AKT, key components of the PI3K pathway. Pharmacologic inhibition using PI3K inhibitors (BEZ235, AEW541), mTOR inhibitors (RAD001), or EGFR/HER2 inhibitors (lapatinib) suppresses the proliferation of letrozole-resistant cells [9]. Targeting PI3K not only enhances ER activity but also improves the efficacy of endocrine therapies. Notably, the PI3K inhibitor alpelisib (BYL719) combined with fulvestrant shows robust antitumor effects in both in vitro and in vivo models [10].

FOXA1 expression

FOXA1 is a critical transcriptional cofactor for ER and androgen receptor (AR). Induction of FOXA1 in breast cancer cells, for example via doxycycline, correlates with upregulation of proliferation-related genes and downregulation of estrogen-responsive genes, thereby promoting tumor aggressiveness and endocrine resistance [11].

Additional genomic and non-genomic mechanisms are under investigation, including progesterone receptor signaling, IGF-IR and FGFR pathways, PARP, MAPK/ERK, c-SRC kinase, STATs, NF-κB, hypoxia-inducible factors, stem cell populations, oxidative stress, drug metabolism, immune modulation, miRNAs, and extracellular vesicles, though many of these processes remain incompletely understood. The complexity is further compounded by the coexistence of multiple resistance mechanisms across different tumor subclones in patients with advanced breast cancer, which may not be detectable from a single metastatic biopsy. Rizavi *et al*. [1] propose that endocrine-resistant breast cancer is evolving into a distinct taxonomy, with some alterations arising due to selective pressure from therapy. Consequently, large-scale genomic analyses of well-characterized patient cohorts are essential to fully delineate the intricate mechanisms underlying ET resistance in HR+, HER2− advanced breast cancer.

Cell-cycle regulators and endocrine resistance

Progression through the cell cycle depends on D-type cyclins, which trigger phosphorylation of retinoblastoma (Rb) protein and activate E2F-mediated transcription of genes such as cyclins A and E, essential for cell-cycle advancement. CDK4 and CDK6 are central in controlling the G1-to-S phase transition, a critical step for both normal and malignant cell proliferation [12].

Preclinical evidence shows that CDK4/6 inhibitors have pronounced activity in ER-positive breast cancer, particularly when combined with anti-estrogen agents. In vitro studies with the CDK4 inhibitor PD-0332991 demonstrated suppression of tumor growth in fulvestrant-resistant ER+ cell lines, as well as in mouse models implanted with ER+ tumors [13]. Transcriptome profiling of 58 tumors from letrozole-resistant patients revealed enrichment of cell-cycle genes, which were significantly downregulated by palbociclib compared to fulvestrant [14].

Abemaciclib acts as a highly selective, reversible CDK4/6 inhibitor, with IC50 values of 2 nM for CDK4 and 10 nM for CDK6 [15]. Similarly, ribociclib has shown strong antitumor effects in ER+ mouse models, both alone and in combination with letrozole, fulvestrant, or PI3K inhibitors.

While palbociclib, ribociclib, and abemaciclib exhibit largely overlapping mechanisms against tumor cells, they may also affect other cell populations, such as those within the bone microenvironment, due to off-target kinase effects. Variations in these off-target activities may partly explain differences in clinical outcomes across the three agents, although their impact on overall disease management remains uncertain [16].

Clinical implications

According to the 4th ESO-ESMO international consensus, primary endocrine resistance is defined as relapse during the first two years of adjuvant ET or progression within six months of starting first-line ET in advanced breast cancer. Secondary endocrine resistance refers to relapse after the first two years of adjuvant ET, relapse within 12 months after completing adjuvant ET, or progression six months or more after initiating ET for advanced disease. These definitions were established prior to widespread use of CDK4/6 inhibitors and achieved only 67% expert agreement.

In phase 3 trials of CDK4/6 inhibitors, patients are generally grouped based on endocrine sensitivity/resistance into four scenarios: (1) de novo metastatic disease; (2) late relapse; (3) early relapse; and (4) second-line therapy **(Table 1)**.

Table 1. CDK4/6 inhibitors phase 3 trials according to endocrine sensitivity/resistance patients representation and outcome results.

Drug	Trial	Setting	Endocrine Sensitivity/Resistance (%)				Efficacy		Adverse Events of Interest
			De Novo	Late Relapse	Early Relapse	Second Line	PFS (Months)	OS (Months)	

Ribociclib	MONALEESA-2 [17]	First line	34	64.7	—	—	RIBO + LET: 25.3 PBO + LET: 16.0 (HR, 0.56; 95% CI, 0.43–0.72; p < 0.001)	Immature	• G ¾ neutropenia: 62% • Diarrhea: 2.4%; • TE: 0.6%; QTcF prolongation: 3.6%
	MONALEESA-7 [18]	First and second line	40	52.5	—	14 (after CT)	RIBO + TAM/NSAI: 23.8 months PBO + TAM/NSAI: 13.0 months (HR, 0.55; 95% CI, 0.44–0.69; p < 0.0001)	HR, 0.712; 95% CI, 0.535–0.948; p = 0.00973	• G 3/4 neutropenia: 60.6% • Diarrhea: 1% • TE: NR QTc prolongation: 7%
	MONALEESA-3 [19]	First and second line	20	29	28	20	RIB + FUL: 20.5 (33.6 in first line) PBO + FUL: 12.8 (19.2 in first line) (HR, 0.593; 95% CI, 0.480–0.732; p < 0.001)	HR, 0.724; 95% CI, 0.568–0.924; p = 0.00455	• G 3/4 neutropenia: 53.4% • Diarrhea: 0.6% • TE: NR QTcF prolongation: 5.6%
Abemaciclib	MONARCH-3 [20]	First line	41.2	58.8	—	—	ABE + NSAI: 28.18 months PBO + NSAI: 14.76 months (HR, 0.540; 95% CI, 0.418–0.698; p = 0.000002)	Immature	• G 3/4 neutropenia: 23.9% • Diarrhea: 82.3% • TE: 4.9% QTcF prolongation: 0.3%
	MONARCH-2 [21]	Second line	—	—	60	38	ABE + FUL: 16.4 PBO + FUL: 9.3 (HR, 0.553; 95% CI, 0.449–0.681; p < 0.001)	HR, 0.757; 95% CI, 0.606–0.945; p = 0.0137	• G 3/4 neutropenia: 23.9% • Diarrhea: 82.3% • TE: 0.9% QTcF prolongation: 0.3%

Palbociclib	PALOMA -2 [22]	First line	37.6	40.01	—	—	PAL + LET: 24.8 PBO + LET: 14.5 (HR, 0.58; 95% CI, 0.46–0.72; p <0.001)	Immature	• G 3/4 neutropenia: 66.4% • Diarrhea: 1.4% • TE: 0.9% QTcF prolongation: 0%
	PALOMA -3 [23–25]	Second line	—	—	21	79	PAL + FUL: 9.5 PBO + FUL: 4.6 (HR, 0.46; 95% CI, 0.36–0.59; p < 0.0001)	HR, 0.81; 95% CI, 0.64–1.03; p = 0.09	• G 3/4 neutropenia: 62% • Diarrhea: 0% • TE: 1.7% QTcF prolongation: <1%

Abbreviations: AE, adverse event; PFS, progression-free survival; HR, hazard ratio; RIBO, ribociclib; LET, letrozole; PBO, placebo; FUL, fulvestrant; PAL, palbociclib; TAM, tamoxifen; NSAI, nonsteroidal aromatase inhibitor; TE, thromboembolic event.

First-line therapy

Current international guidelines recommend endocrine therapy (ET) as the first-line treatment for HR+, HER2− advanced breast cancer (ABC), even in patients presenting with visceral involvement. Chemotherapy is generally reserved for cases of visceral crisis, defined by severe organ dysfunction and rapid disease progression, or for patients who progress on multiple lines of ET. A recent meta-analysis indicated that no chemotherapy regimen, with or without targeted therapy, outperforms the combination of CDK4/6 inhibitors with hormone therapy in terms of progression-free survival (PFS) [26]. Given the substantial improvement in outcomes when CDK4/6 inhibitors are added to standard hormonal therapy compared with ET alone, this combination is increasingly considered the standard of care for first- or second-line treatment **(Table 1)**. A network meta-analysis comparing CDK4/6 inhibitors combined with aromatase inhibitors (AIs) or fulvestrant versus AI or fulvestrant monotherapy confirmed that CDK4/6 inhibitors are equally effective with AI in the first-line setting and consistently outperform monotherapy regardless of patient or tumor characteristics [27].

In treatment-naïve patients, all three approved CDK4/6 inhibitors improved PFS compared with ET alone (MONALEESA-2: HR 0.45; MONALEESA-3: HR 0.57; MONALEESA-7: HR 0.43; MONARCH-3: HR 0.54; PALOMA-2: HR 0.67) [17–20, 22]. MONALEESA-7 uniquely evaluated first-line CDK4/6 therapy in premenopausal women, whereas premenopausal participants in palbociclib and abemaciclib trials (PALOMA-3 and MONARCH-2, second-line studies) accounted for 20.7% and 16.1%, respectively, receiving combination therapy with fulvestrant. Updated MONALEESA-7 results demonstrated that ribociclib plus ET significantly improved overall survival (OS) compared with ET alone, with estimated 42-month OS rates of 70.2% versus 46.0% (HR 0.71; p = 0.00973) [28]. Based on these findings, ribociclib in combination with ET (AI or tamoxifen) plus ovarian function suppression (OFS) has been approved by the Italian Medicines Agency (AIFA) as a preferred first-line option for premenopausal HR+, HER2− ABC patients.

Objective response rates (ORR) were similar across trials: MONALEESA-2 (52.7%), MONARCH-3 (59.2%), and PALOMA-2 (55.3%). However, ribociclib plus fulvestrant in MONALEESA-3 yielded a longer median PFS (33.6 months) versus 22–28 months with palbociclib or abemaciclib plus AI, and showed a 28% relative reduction in risk of death in the first-line setting, suggesting it may be the preferred regimen for postmenopausal patients [19]. Data on OS for palbociclib and abemaciclib remain immature or largely derived from retrospective real-world studies [29, 30], whereas ribociclib OS data come directly from phase 3 trials [18,19].

All three CDK4/6 inhibitors exhibit favorable safety profiles. Grade 3–4 neutropenia was more common with ribociclib and palbociclib, whereas abemaciclib was associated with diarrhea and abdominal pain **(Table 1)**. QTcF prolongation with ribociclib occurred in no more than 7% of patients in MONALEESA-7, with even lower incidence in real-world practice. Dose reductions due to adverse events occurred in 54.5%, 33.1%, and 31% of patients in MONALEESA-2, MONALEESA-3, and MONALEESA-7, respectively, without compromising PFS [17–19]. Thus, ribociclib dosage can be adjusted for toxicity without loss of efficacy. Similarly, MONARCH [20, 21] and PALOMA-3 [24] demonstrated that dose modifications did not affect PFS outcomes.

The clinical landscape is further complicated by potential drug–drug interactions (DDIs) in patients receiving CDK4/6 inhibitors, particularly in the context of polypharmacy [31]. Understanding how patient metabolism and DDIs influence both safety and efficacy is critical for personalizing therapy in ABC **(Figure 2)**. Concomitant medications, pharmacogenomic profiles, and pathophysiological factors can affect the pharmacokinetics and pharmacodynamics of CDK4/6 inhibitors. Personalized strategies that integrate these variables may optimize the safe and effective use of these agents [32]. In line with precision medicine approaches, our institution is investigating Drug-PIN, a software platform that combines DDI data with patient pharmacogenomic profiles to guide individualized cancer treatment [33].

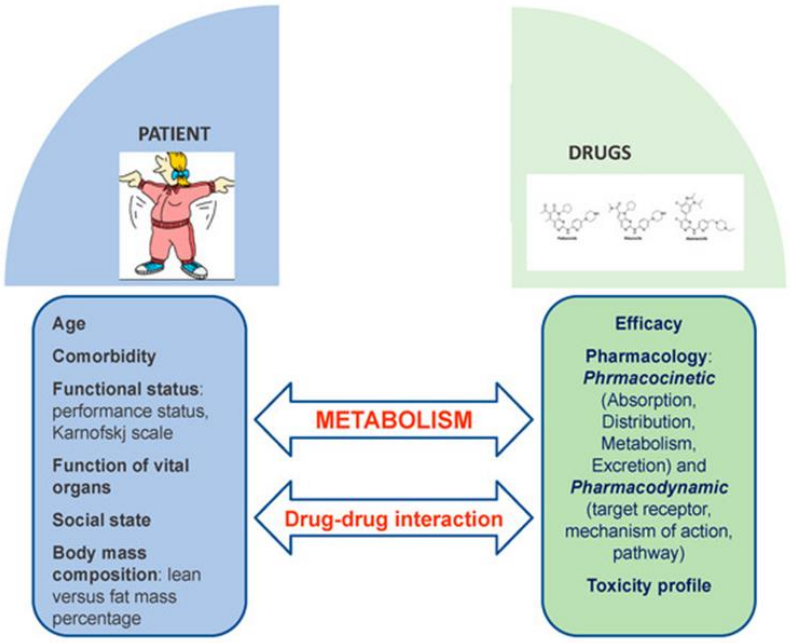

Figure 2. Complexity of Metabolism and Potential Drug–Drug Interactions

Second-line therapy and early relapse

The AIOM guidelines classify early relapse as aggressive disease that emerges shortly after adjuvant therapy—either during treatment or within 12 months of its completion. Although this differs slightly from the ESMO definitions of primary and secondary endocrine resistance, it aligns with the criteria applied in three pivotal CDK4/6 inhibitor trials:

- MONALEESA-3: Relapse occurring during or within 12 months after finishing adjuvant or neoadjuvant ET.
- MONARCH-2: Progression on neoadjuvant or adjuvant ET within 12 months post-treatment.
- PALOMA-3: Relapse or progression during treatment or within 12 months after completing adjuvant ET, irrespective of menopausal status.

These trials included patients whose disease progressed following single-agent ET (tamoxifen or aromatase inhibitor), with the main goal being to compare survival between fulvestrant combined with a CDK4/6 inhibitor versus fulvestrant plus placebo. Apart from MONALEESA-3, MONARCH-2, and PALOMA-3 enrolled both pre- and postmenopausal women [19, 21, 23, 24].

According to the ABC 4 (4th ESO-ESMO International Consensus) guidelines [34], premenopausal women with ER+ ABC should first undergo ovarian suppression or ablation (OFS/OFA) to achieve a postmenopausal hormonal state before receiving endocrine therapy, with or without targeted agents, so that their treatment is consistent with that of postmenopausal patients. Ovarian ablation via laparoscopic bilateral oophorectomy offers permanent estrogen suppression, acts as contraception, avoids the initial tumor flare seen with luteinizing hormone-releasing hormone agonists, and may increase eligibility for clinical trials.

In the PALOMA-3 trial, pre/perimenopausal patients (n = 108; 21%) did not show a significant OS difference compared with older patients (HR 1.07; 95% CI 0.61–1.86) [35]. Similarly, MONARCH-2 (114 premenopausal patients; 17%) reported no OS difference (HR 0.68; 95% CI 0.37–1.25) [21]. By contrast, the MONALEESA-7

trial demonstrated that ribociclib significantly improved both PFS and ORR compared with placebo in premenopausal women who had received prior chemotherapy for ABC [36].

When indirectly comparing the three phase 3 trials of CDK4/6 inhibitors plus fulvestrant **(Table 2)**, PFS outcomes were largely comparable, while OS varied slightly. These discrepancies may reflect differences in the pharmacokinetics of ribociclib, palbociclib, and abemaciclib [31] as well as variations in patient characteristics across the studies **(Table 2)**.

Table 2. Phase 3 trials of CDK4/6 inhibitors including patients in second-line and early relapse settings, relative to the total enrolled population (N)

Trial	Population (Subgroup)	n/N	Intervention	PFS (Months)	OS (Months)	ORR (%)
MONALEESA-3	Postmenopausal women and men; second-line or early relapse subgroup	346/726	Ribociclib + fulvestrant vs placebo + fulvestrant	14.6 (HR 0.57)	40.2 (HR 0.73)	40.9
MONALEESA-7	Premenopausal women; early relapse or prior first-line chemotherapy	94/672	Ribociclib + ET + goserelin vs placebo + ET + goserelin	16.6 (HR 0.54)	Not reached (HR 0.67)	26
MONARCH-2	Pre- and postmenopausal women and men; second-line or early relapse; primary/secondary ET resistance	669	Abemaciclib + fulvestrant vs placebo + fulvestrant	16.4 (HR 0.55)	46.7 (HR 0.75)	48.1
PALOMA-3	Pre- and postmenopausal women and men; early relapse/second or later lines; ET sensitivity yes/no	521	Palbociclib + fulvestrant vs placebo + fulvestrant	11.2 (HR 0.50)	34.9 (HR 0.81; NS, p = 0.09)	25

Abbreviations: PFS, progression-free survival; OS, overall survival; ORR, overall response rate; HR, hazard ratio; ET, endocrine therapy; NS, not significant.

For instance, PALOMA-3 enrolled patients who had previously received multiple lines of therapy, unlike MONALEESA-3 and MONARCH-2, which allowed only a single prior line of endocrine therapy [19, 21, 23]. Across patients with prior ET exposure, median PFS consistently favored the CDK4/6 inhibitor arms in MONALEESA-3, MONARCH-2, and PALOMA-3. Specifically, postmenopausal women who had received prior ET or had early relapse experienced a median OS of 40.2 months with ribociclib compared to 32.5 months with placebo (HR 0.73; 95% CI, 0.53–1.00) in MONALEESA-3 [19].

In MONARCH-2, patients in the early relapse subgroup achieved a median OS of 46.7 months with abemaciclib versus 37.3 months with placebo, representing an improvement of 9.4 months (HR 0.757; 95% CI, 0.606–0.945; p = 0.01) [21]. Notably, the survival curves separated earlier, and the effect was more pronounced in patients with primary endocrine resistance (HR 0.686; 95% CI, 0.451–1.043) compared to those with secondary resistance (HR 0.787; 95% CI, 0.606–1.021), although the interaction was not statistically significant.

In PALOMA-3, median OS was not significantly different overall (34.9 months with palbociclib-fulvestrant vs 28.0 months with placebo-fulvestrant; HR 0.81; 95% CI, 0.64–1.03; p = 0.09) [24], likely reflecting the inclusion of patients irrespective of menopausal status and those treated with more than one prior line of ET. A statistically significant OS benefit (39.7 vs 29.7 months; HR 0.72) was observed only among patients with documented sensitivity (secondary resistance) to prior ET.

From these findings, several observations emerge: (1) ribociclib appears more effective in patients with acquired resistance; (2) abemaciclib demonstrates stronger activity in the primary endocrine resistance setting; (3) palbociclib shows promising results in pretreated patients, though OS data remain less definitive. Across all three trials, CDK4/6 inhibitors conferred benefit regardless of the presence of visceral metastases.

Retrospective analyses also provide insights into therapy following CDK4/6 inhibitor progression. In one real-world study of 104 patients who progressed on palbociclib, the most common subsequent therapies were capecitabine (n = 21), eribulin (n = 16), nab-paclitaxel (n = 15), and exemestane plus everolimus (n = 12). Median PFS after first-, second-, or later-line palbociclib therapy was 17.0, 9.3, and 4.2 months with hormonal therapy or

combinations (n = 32; p = 0.04), versus 4.7, 4.1, and not reached months with chemotherapy (n = 70; p = 0.56), indicating that hormone-based therapy remains a viable option post-palbo progression [37].

Similarly, in a retrospective BOLERO-2 analysis, 17 patients had prior CDK4/6 therapy and 16 had not. No significant differences were observed in PFS (5.7 vs 4.7 months; p = 0.890) or OS (17.8 vs 11.4 months; p = 0.177), suggesting that everolimus plus exemestane continues to be an effective option in later treatment lines [38].

Another retrospective study examined 58 HR+/HER2− ABC patients who received abemaciclib following palbociclib progression. Of these, 20 patients (34%) underwent sequential CDK4/6 therapy, while 38 (66%) had at least one intervening non-CDK4/6 regimen. Fourteen patients (24%) received abemaciclib monotherapy, and 44 (76%) received it with anti-estrogens, including fulvestrant (52%), aromatase inhibitors (22%), or tamoxifen (2%) [39]. Early disease progression (<90 days) occurred in 20 patients (34%), whereas 21 patients (36%) remained on treatment for more than six months, with 10 still receiving therapy at interim analysis (181–413 days). Median PFS was 5.8 months (95% CI, 3.4–8.0). While limited, these results indicate that a meaningful proportion of patients may still benefit from switching to another CDK4/6 inhibitor after prior CDK4/6 therapy.

Future perspectives

Currently, it remains unclear which combinations of ET and targeted agents are optimal, how they compare to single-agent chemotherapy, or which patients are best suited for specific CDK4/6 inhibitors. A deeper understanding of the molecular mechanisms driving CDK4/6 inhibitor resistance is urgently needed.

Investigations are ongoing into potential resistance mechanisms and biomarkers [40], which can be broadly classified into two categories:

1. Cell-cycle-specific mechanisms: loss of retinoblastoma protein (RB), CCND1 amplification or p16 loss, CCNE1/2 amplification or overexpression, CDK4/6 amplification or overexpression [41–43].
2. Cell-cycle-nonspecific mechanisms: activation of the PIK3CA or FGFR pathways, overexpression of MDM2, ESR1 mutation or altered expression, PD-1 expression, thymidine kinase-1 (TK1) activity, FAT1 loss [41], and activation of autophagy [44].

A schematic overview of the main mechanisms implicated in CDK4/6 inhibitor resistance is provided in **Figure 3**.

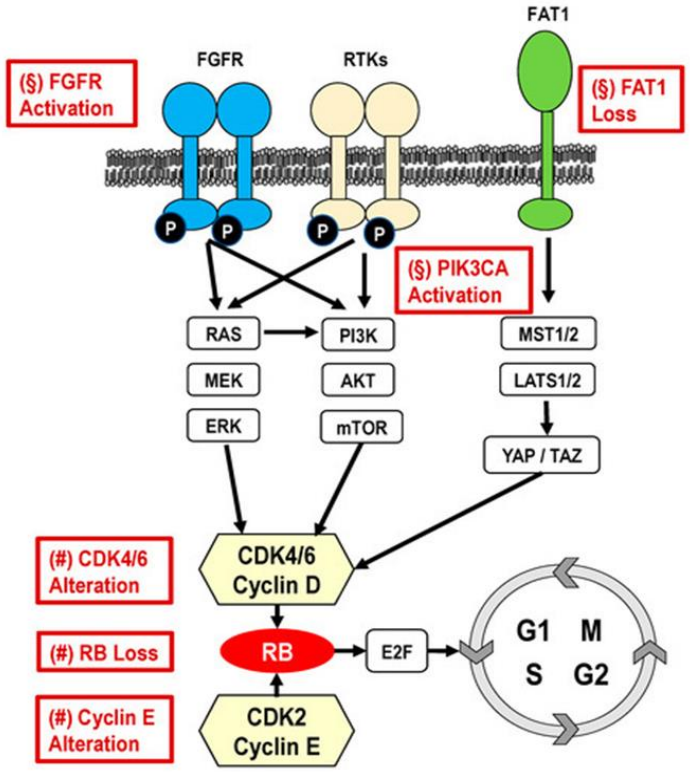

Figure 3. Overview of the principal mechanisms potentially driving resistance to CDK4/6 inhibitors: (§) Cell cycle–non-specific; (#) Cell cycle–specific.

Cell cycle–specific mechanisms of CDK4/6 inhibitor resistance

- RB loss

RB, a tumor suppressor, is the primary target of CDK4/6 activity. Preclinical studies indicate that loss of RB can drive resistance to CDK4/6 inhibitors, although its occurrence in ER+ breast cancer is relatively rare (<5%). In PALOMA-3, polyclonal RB1 mutations were detected in 4.7% of patients in the palbociclib arm, with no significant interaction between RB1 expression (or CDK4/6 and cyclin D1 levels) and treatment outcome [24]. Similarly, in PALOMA-2, no association was observed between RB status—evaluated by immunohistochemistry, FISH, or gene expression—and response to therapy [45]. Overall, RB expression assessed by immunohistochemistry was positive in 90.9% of patients and negative in 9.1%. Exploratory analysis showed a consistent benefit of palbociclib plus letrozole in RB-positive tumors (HR 0.543; $p < 0.0001$), whereas RB-negative tumors did not exhibit a meaningful response (HR 0.868; $p = 0.698$), though the small number of RB-null patients limits conclusions.

- CCND1 amplification / p16 loss

Amplification of CCND1 (≈15% of breast cancers) and loss of the tumor suppressor p16INK4A have been proposed as contributors to CDK4/6 inhibitor resistance. However, the PALOMA-1 study did not demonstrate any significant PFS difference in patients with CCND1 amplification or p16 loss compared with the general population [22]. Similarly, cyclin D1 expression was not associated with palbociclib benefit in PALOMA-2 or PALOMA-3, and p16 loss did not correlate with treatment outcome [24, 45].

- CCNE1/2 amplification or overexpression

CCNE1, which encodes cyclin E1, has been shown in preclinical models to be upregulated in CDK4/6 inhibitor–resistant tumors [46]. Nonetheless, in MONALEESA-2, mRNA analysis of tumor samples from 391 of 668 patients revealed that ribociclib plus letrozole improved PFS regardless of CCNE1 expression [17]. Likewise, PALOMA-2 found no association between CCNE1/2 expression (assessed via IHC, FISH, or gene expression) and benefit from palbociclib [45]. In PALOMA-3, mRNA profiling of 302 patients demonstrated that all biomarker-defined groups derived benefit, but patients with low CCNE1 expression showed a greater response (median PFS: 14.1 vs 4.8 months) than those with high expression (7.6 vs 4.0 months). The predictive value of CCNE1 was stronger in metastatic biopsies (interaction $p < 0.001$) than in archived primary samples (interaction $p = 0.09$) [35], a pattern also observed in MONARCH-2 [47].

- CDK4/6 amplification or overexpression

Overexpression of CDK4 or CDK6 has been associated with resistance in preclinical models. Clinically, PALOMA-2 data indicated that high CDK4 expression predicted faster progression with placebo plus letrozole, but not with palbociclib plus letrozole [45]. CDK6 expression did not show a similar impact (low expression: HR 0.596; high expression: HR 0.592). In MONALEESA-2, ribociclib addition to letrozole appeared slightly more effective in patients with elevated expression of cell-cycle regulatory genes (HR 0.45 vs 0.66). In the placebo group, high expression of these genes correlated with shorter median PFS. The genes analyzed included CCNA2, CCND1-3, CCNE1, CDK2, CDK4/6, CDKN1A/B, CDKN2A-C, RB1, E2F1/3, TFDP1, and TP53.

Cell cycle–non-specific mechanisms of resistance to CDK4/6 inhibitors

- PIK3CA pathway activation

In the MONALEESA-7 trial, ctDNA sequencing of 565 patients revealed PIK3CA mutations in 28% of cases [48]. Patients with wild-type PIK3CA appeared to derive a slightly higher PFS benefit from ribociclib than those with mutated PIK3CA, although the interaction was not statistically significant. Likewise, baseline ctDNA biomarker analyses from MONALEESA-3 and MONARCH-2 confirmed consistent benefit from ribociclib and abemaciclib, respectively, independent of PIK3CA mutation status [49, 50]. In MONALEESA-2, mRNA expression of PI3K pathway genes (AKT1/2, PIK3CA, PTEN) indicated that ribociclib improved PFS regardless of high or low expression levels [17]. PALOMA-3 baseline ctDNA analysis (395 patients) identified PIK3CA mutations in 33%, but these did not correlate with outcomes [25]. Further ctDNA sequencing (195 patients) showed that 7.7% acquired PIK3CA mutations during treatment, but acquisition rates were similar between treatment arms [51]. Therefore, PIK3CA cannot be considered a reliable predictive biomarker for CDK4/6 inhibitor response. MONALEESA-3 analyses similarly confirmed ribociclib benefit irrespective of baseline PIK3CA alterations.

- FGFR pathway activation

Preclinical studies suggest that alterations in FGFR/FGF signaling may drive fulvestrant resistance and confer cross-resistance to palbociclib [52]. In PALOMA-2, tumors with higher FGFR2 expression seemed to gain more PFS benefit from palbociclib plus letrozole, although this interaction was not statistically significant [45]. FGFR signaling may also carry prognostic value: in MONALEESA-2, patients with FGFR1 amplification (5%) showed shorter PFS than those with wild-type FGFR1 [53], and PALOMA-3 ctDNA analysis linked FGFR1 amplification to early progression risk [54].

- MDM2 overexpression

MDM2 inhibits p53 activity and disrupts cellular senescence, which may contribute to CDK4/6 inhibitor resistance. Preclinical data indicate that combining MDM2 inhibition with CDK4/6 blockade yields synergistic effects [55], suggesting MDM2 inhibitors could overcome such resistance [56].

- ESR1 expression and mutation

ESR1 levels and mutations mainly serve as prognostic indicators in ER-targeted therapy. In MONALEESA-2, ribociclib benefit appeared greater in patients with high ESR1 expression, though longer PFS trends were seen in both treatment arms [17]. PALOMA-2 showed similar trends with high ESR1 levels [45]. PALOMA-3 and MONARCH-2 demonstrated palbociclib benefit regardless of ESR1 mutation status [50, 51]. Consequently, ESR1 cannot be deemed predictive for CDK4/6 inhibitor efficacy.

- PD-1 expression

Lower PD-1 expression in PALOMA-2 correlated with greater PFS benefit from palbociclib plus letrozole [45], suggesting PD-1 signaling may limit CDK4/6 inhibitor efficacy. Ongoing trials are exploring combinations with PD-1 immune checkpoint inhibitors.

- Thymidine kinase-1 (TK1)

TK1, an enzyme in the pyrimidine salvage pathway crucial for DNA synthesis, serves as a prognostic marker for ET sensitivity. Elevated TK1 activity correlates with poor prognosis in primary breast cancer. Pilot studies indicate patients with lower baseline plasma TK1 activity have longer median PFS on ET [57], and preclinical work suggests TK1 may be an early indicator of proliferation inhibition in response to palbociclib [58].

- FAT1 loss

FAT1, a tumor suppressor commonly mutated in cancers, has been linked to resistance to CDK4/6 inhibitors. Genomic analysis of 348 ER+ breast cancers showed that FAT1 loss increases CDK6 expression via the Hippo pathway, diminishing sensitivity to these drugs [59].

- Autophagy activation

Evidence from preclinical models suggests autophagy can counteract the cell-cycle arrest induced by CDK4/6 inhibitors, potentially contributing to resistance. Inhibition of autophagy may therefore enhance CDK4/6 inhibitor efficacy and help overcome resistance [60].

Additional mechanisms underlying resistance

The BIOITALEE study is currently investigating ctDNA changes throughout treatment to determine how these molecular alterations may influence patient outcomes [61]. Early analyses revealed several baseline tumor alterations linked to clinical characteristics and therapy response. Notably, copy number gains in FGFR1–3 were more commonly observed in patients with visceral metastases. Alterations in MYC or disruptions in the ER nuclear signaling pathway were frequently present in tumors that were progesterone receptor–positive with Ki67 ≥ 14%. Furthermore, MYC amplification, TP53 mutations, and abnormalities in HER and CDK4/6 pathways correlated with disease progression at the first tumor assessment (10% of cases), suggesting these alterations could represent intrinsic mechanisms of resistance to the combination of ribociclib and letrozole.

Post-CDK4/6 inhibitor therapeutic strategies and emerging combinations

For patients whose disease advances despite CDK4/6 inhibitor therapy, new treatment approaches are under investigation, as outlined in **Tables 3 and 4**, including strategies that combine endocrine therapy with novel targeted agents.

Table 3. Ongoing studies based on CDK4/6 inhibitors beyond progression.

Trial	Phase	Study Arms	Previous CDK4/6
MAINTAIN (NCT02632045)	2	Ribociclib + fulvestrant versus placebo + fulvestrant	AI + palbociclib/ribociclib

NCT02738866	2	Palbociclib + fulvestrant	Palbociclib + AI
PACE (NCT03147287)	2	Fulvestrant versus fulvestrant + palbociclib versus ± avelumab	CDK4/6 inhibitor-based regimen
NCT02871791	½	Palbociclib + everolimus + exemestane	CDK4/6 inhibitor-based regimen
TRINITI-1 (NCT02732119)	1/2	Ribociclib + everolimus + exemestane	CDK4/6 inhibitor-based regimen
NCT01857193	1B	Ribociclib + exemestane versus ± everolimus	Naive or refractory to CDK4/6-inhibitor
PALMIRA (NCT03809988)	2	Palbociclib + ET	Had clinical benefits with palbociclib + ET in first line

Abbreviations: AI, aromatase inhibitor; ET, endocrine therapy.

Table 4. New agents after CDK4/6 inhibitor progression.

Trial	Phase	Study Arms	Previous CDK4/6
CDK7 inhibitor after CDK4/6 inhibitor progression			
NCT03134638	1	SY-1365 + fulvestrant	CDK inhibitor + AI
NCT03363893	1	CT-7001 + fulvestrant	CDK inhibitor
CDK2 inhibitor after CDK4/6 inhibitor progression			
NCT03519178	1/2A	PF-06873600 versus ± ET	CDK inhibitor + ET
Selective estrogen receptor downregulator (SERD) (elacestrant) after CDK4/6 inhibitor progression			
EMERALD (NCT03778931)	3	Elacestrant	CDK inhibitor + AI or fulvestrant
BCL-2 inhibitor (venetoclax) afterCDK4/6 inhibitor progression			
VERONICA (NCT03519178)	2	Venetoclax + fulvestrant	CDK inhibitor-based regimen
Fibroblast growth factor receptor (FGFR) inhibitor after CDK4/6 inhibitor progression			
NCT03238196	1	Fulvestrant + palbociclib + erdafitinib	Previous palbociclib allowed
Immune checkpoint inhibitor after CDK4/6 inhibitor progression			
PACE (NCT03147287)	2	Fulvestrant versus fulvestrant + palbociclib versus ± avelumab	CDK4/6 inhibitor-based regimen
NCT0329469	1	Fulvestrant + ribociclib + PDR001	Not specified
MORPHEUS HR+BC (NCT03280563)	1/2	Fulvestrant Atezolizumab + entinostat Atezolizumab + fulvestrant Atezolizumab + ipatasertib Atezolizumab + ipatasertib+ fulvestrant Atezolizumab + bevacizumab + ET Atezolizumab + abemaciclib + fulvestrant	CDK4/6 inhibitor in first or second line

Abbreviations: AI, aromatase inhibitor; ET, endocrine therapy.

The principal approaches can be classified into four categories: (1) continue the CDK4/6 inhibitor and switch endocrine therapy (ET); (2) continue ET and switch to a different CDK4/6 inhibitor or another targeted therapy; (3) maintain both ET and CDK4/6 inhibitor while targeting an alternative pathway; and (4) explore new therapeutic agents. In early findings from the phase 1/2 TRINITI-1 trial involving men and postmenopausal women with HR+, HER2− locally advanced or metastatic breast cancer who progressed on a CDK4/6 inhibitor, a triplet regimen of ribociclib combined with everolimus and exemestane achieved a 39% clinical benefit at 24 weeks [48], demonstrating both efficacy and tolerability of continuous ET plus mTOR inhibitor plus CDK4/6 inhibitor therapy. Approximately 40% of HR+, HER2− advanced breast cancers harbor activating PIK3CA mutations; based on the phase 3 SOLAR-1 study, alpelisib combined with fulvestrant represents a potential option for patients with PIK3CA-mutant tumors who progressed after prior ET (with or without CDK4/6 inhibitors), showing a median progression-free survival of 11.0 months versus 5.7 months in wild-type cases (hazard ratio

0.65; p = 0.00065) [62]. Alpelisib has now been approved in combination with fulvestrant for postmenopausal women and men with HR+, HER2− advanced breast cancer harboring PIK3CA mutations after progression on ET monotherapy; however, the optimal strategy for patients who progress after first-line CDK4/6 inhibitor plus ET remains unclear. The ByLieve phase 2 multicenter, open-label study, evaluating alpelisib plus fulvestrant or letrozole in patients progressing after CDK4/6 inhibitor-based therapy or with limited prior chemotherapy, uses 6-month progression-free survival as its primary endpoint, and early interim results suggest a potential advantage for the fulvestrant cohort, although data are still immature [63]. Ultimately, considering the potential emergence of driver PIK3CA mutations following prior ET plus CDK4/6 inhibitor therapy, combining PI3K or FGFR inhibitors with CDK4/6 inhibitors—or other targeted agents in triplet regimens—emerges as a promising approach to overcome resistance to single-agent CDK4/6 inhibitors in early-phase HR+, HER2− breast cancer **(Table 5)**.

Table 5. Ongoing studies of multiple combination treatments with CDK4/6 inhibitors.

Trial	Treatment Arms	Phase	Study Population	Primary Endpoint	Status
PASTOR: Investigation of vistusertib combined with palbociclib in individuals with ER-positive metastatic breast cancer	Vistusertib (mTOR inhibitor) + palbociclib + fulvestrant Placebo + palbociclib + fulvestrant	1/2	Postmenopausal patients with ER+ locally advanced or metastatic breast cancer previously treated with endocrine therapy	PFS	Completed; results not yet available
Evaluation of copanlisib, letrozole, and palbociclib for hormone receptor-positive, HER2-negative breast cancer (stages I–IV)	Copanlisib (PI3K inhibitor) + letrozole Copanlisib + letrozole + palbociclib	1b/2	Postmenopausal women with ER+/HER2− breast cancer at any stage	Ki-67 modification, dose-limiting toxicity	Enrolling participants
IPATunity150: Comparison of ipatasertib plus palbociclib and fulvestrant versus placebo plus palbociclib and fulvestrant in HR-positive, HER2-negative locally advanced unresectable or metastatic breast cancer	Ipatasertib (AKT inhibitor) + palbociclib + fulvestrant versus placebo + palbociclib + fulvestrant	1b/3 (randomized)	HR+ HER2− advanced breast cancer with progression during adjuvant endocrine therapy or within 12 months of first-line endocrine therapy	PFS	Enrolling participants
Ribociclib combined with everolimus and exemestane for postmenopausal women with HR-positive, HER2-negative locally advanced or metastatic breast cancer	Ribociclib + exemestane + everolimus versus ribociclib + exemestane	Nonrandomized, two parallel arms	Disease recurrence during or within 12 months after adjuvant letrozole/anastrozole, or progression during or within 1 month after letrozole/anastrozole for advanced disease	Dose-limiting toxicity and disease control rate	Completed
LEE011 paired with fulvestrant and either alpelisib or buparlisib in postmenopausal women with HR-positive, HER2-negative locally recurrent or advanced metastatic breast cancer	LEE011 + fulvestrant + alpelisib or LEE011 + fulvestrant + buparlisib or LEE011 + fulvestrant	1b/2 (nonrandomized)	HR+ HER2− locally recurrent or advanced metastatic breast cancer	Dose-limiting toxicity / PFS	Completed

Assessment of gedatolisib tolerability and efficacy when combined with palbociclib/letrozole or palbociclib/fulvestrant in women with metastatic breast cancer	Gedatolisib + palbociclib/letrozole or gedatolisib + palbociclib/fulvestrant	1b (nonrandomized)	HR+ HER2− locally recurrent or advanced metastatic breast cancer	Dose-limiting toxicity, objective response rate	Enrolling participants
Abemaciclib combined with various agents in patients with metastatic breast cancer	Abemaciclib + letrozole or anastrozole or tamoxifen or exemestane or everolimus or trastuzumab or fulvestrant or pertuzumab	1b	Metastatic breast cancer	Dose-limiting toxicity	Active, no longer recruiting
PIPA: Palbociclib plus PI3K inhibitors with optional subsequent fulvestrant in PIK3CA-mutant breast cancer	Palbociclib + taselisib or pictilisib (± fulvestrant)	1b	Advanced solid malignancies; advanced breast cancer: ER+ with progression after ≥1 prior endocrine therapy line, or PIK3CA-mutant breast cancer progressed after ≥1 prior endocrine or chemotherapy line, or treatment-refractory breast cancer	Dose-limiting toxicity, safety profile	Active, no longer recruiting

Abbreviations: MBC, metastatic breast cancer; PFS, progression-free survival; DLT, dose-limiting toxicity; ET, endocrine therapy; DCR, disease control rate; CT, chemotherapy.

Despite significant progress in scientific understanding, the ideal treatment pathway for HR+ advanced breast cancer (ABC) remains unclear; nonetheless, a rational approach should consider (1) prior therapies received, (2) disease burden, (3) patient preferences, and (4) cost and accessibility. Clearly, as first-line treatment increasingly incorporates CDK4/6 inhibitors combined with ET, subsequent therapy strategies must focus on patients who experience progression during or after this combination **(Figure 4)**.

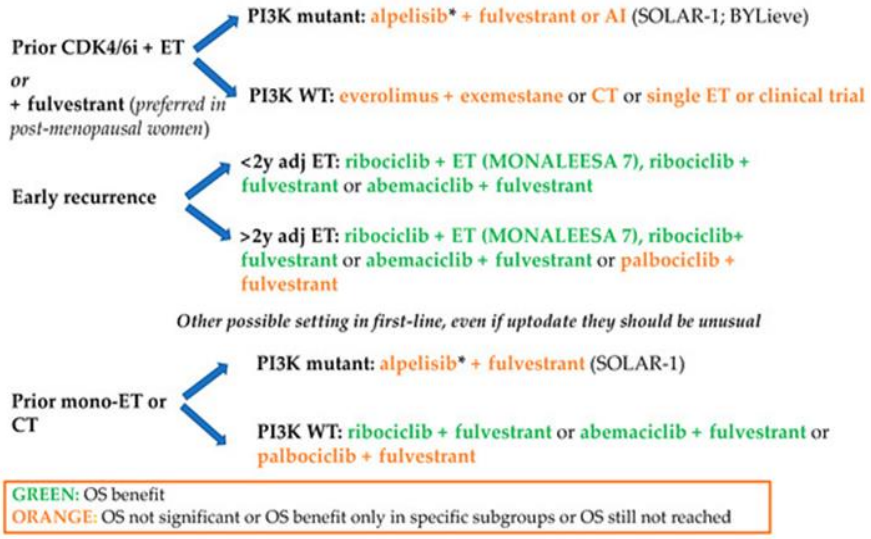

Figure 4. Proposed treatment algorithm for HR+, HER2− advanced breast cancer. Abbreviations: CT, chemotherapy; ET, endocrine therapy with aromatase inhibitor or tamoxifen ± ovarian suppression; AI,

aromatase inhibitor. *Alpelisib is currently approved only for use after disease progression following endocrine therapy as monotherapy.

In addition, the mechanisms underlying resistance to CDK4/6 inhibitors, suggested by preclinical genomic and transcriptomic studies, require further clinical validation in larger patient cohorts and prospective trials. Analyses of single-nucleotide polymorphisms—common genetic variations—may provide additional insights into predicting individual responses to CDK4/6 inhibitors [31].

Body composition may also impact outcomes in patients treated with CDK4/6 inhibitors: in a retrospective study, baseline sarcopenia (skeletal muscle index <40) was linked to significantly shorter progression-free survival (PFS), whereas higher visceral fat index and visceral fat density correlated with improved PFS [64].

Moreover, tumor tissue analysis, still the gold standard, should be complemented with liquid biopsy, as longitudinal monitoring of circulating tumor DNA (ctDNA) can reveal both baseline mutational profiles and emerging genomic resistance mechanisms—potentially actionable—under the selective pressure of CDK4/6 inhibitors.

Conclusion

CDK4/6 inhibitors have transformed the management of HR+/HER2− metastatic breast cancer, improving key clinical outcomes while maintaining a manageable safety profile. This review summarizes their mechanisms of action and clinical implications. However, intrinsic or acquired resistance remains a major cause of disease progression, highlighting the urgent need to understand resistance mechanisms. We discussed potential resistance pathways and emerging strategies aimed at enhancing CDK4/6 inhibitor efficacy to improve survival and quality of life. Personalizing therapy based on baseline genomic profiles and the selective pressures imposed during treatment represents the optimal approach for managing HR+, HER2− ABC. While comprehensive genomic profiling is currently feasible mainly in experimental settings, evaluating single gene mutations, such as PIK3CA, is more practical and immediately relevant because targeted therapies are available and likely to enter routine practice soon. Further research is needed to determine the optimal sequencing of therapies and to assess outcomes of triplet regimens, always balancing efficacy and safety.

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