Review Article

A narrative review about CDK4/6 inhibitors in the setting of drug resistance: updates on biomarkers and therapeutic strategies in breast cancer

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Background and Objective: Previous studies have demonstrated that cyclin-dependent kinase 4/6 (CDK4/6) inhibitors combined with endocrine therapy are able to effectively improve the prognosis of hormone receptor positive (HR^+), human epidermal growth factor receptor 2 (HER2) negative advanced breast cancer (ABC). Five CDK4/6 inhibitors, palbociclib, ribociclib, abemaciclib, dalpiciclib, and trilaciclib have been approved for the treatment of this breast cancer subset at present. The efficacy and safety profile of adding these CDK4/6 inhibitors to endocrine therapies in HR^+ breast cancer has been proved in a number of clinical trials. Besides, extending the application of CDK4/6 inhibitors to $HER2^+$ or triple negative breast cancers (TNBCs) has also led to some clinical benefits.

Methods: A comprehensive, non-systematic review of the latest literature about CDK4/6 inhibitors resistance in breast cancer was conducted. The examined database was PubMed/MEDLINE, and the last search was run on October 1, 2022.

Key Content and Findings: In this review, the generation of CDK4/6 inhibitors resistance is related to gene alteration, pathway dysregulation, and tumor microenvironment change. With a deeper insight in the mechanisms of CDK4/6 inhibitor resistance, some biomarkers have presented the potential to predict drug resistance and showed prognostic value. Furthermore, in preclinical studies, some modified treatment strategies based on CDK4/6 inhibitors exhibited effectiveness on drug-resistant tumors, suggesting a preventable or reversible drug-resistant status.

Conclusions: This review clarified the current knowledge about mechanisms, the biomarkers to overcome the drug resistance of CDK4/6 inhibitors, and the latest clinical progresses about CDK4/6 inhibitors. Possible approaches to overcome CDK4/6 inhibitors resistance were further discussed. For example, using another CDK4/6 inhibitor, PI3K inhibitor, mTOR inhibitor, or a novel drug.

Keywords: Breast cancer; cyclin-dependent kinase4/6 (CDK4/6) inhibitors; resistant markers; hormone receptor-positive; clinical trial

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Introduction

Breast cancer is the most common malignant tumor among female patients, with about 24.5% of all new tumors in women worldwide (1). Hormone receptor positive (HR^+) and human epidermal growth factor receptor 2 negative ($HER2^-$) breast cancer is the most frequently occurring subtype, accounting for approximately 70% of the patients (2). Endocrine therapy was the main treatment for advanced $HR^+/HER2^-$ breast cancer for a while, without major progress in innovative therapies. Until 2015, the treatment of $HR^+/HER2^-$ advanced breast cancer (ABC) entered the era of CDK4/6 inhibitors in combination with endocrine therapy. To date, there have been five CDK4/6 inhibitors (including palbociclib, ribociclib, abemaciclib, dalpiciclib, and trilaciclib), which have been authorized for using in the treatment of $HR^+/HER2^-$ ABC. Their efficacy and safety had been proven in numbers of clinical trials. In the PALOMA-2 trial, $HR^+/HER2^-$ patients who received palbociclib + letrozole had significantly longer progression-free survival (PFS) by 13.1 months (27.6 *vs.* 14.5 months) *vs.* placebo + letrozole. In the MONARCH-3 trial, the median PFS of abemaciclib + non-steroid aromatase inhibitor (NSAI) was 28.2 months and placebo + NSAI arm was 14.8 months. In the MONALEESA-2 trial, the median PFS was 25.3 months for ribociclib + letrozole and 16.0 months for placebo + letrozole. In the MONALEESA-3 trial, median PFS was also significantly improved with ribociclib + fulvestrant *vs.* placebo + fulvestrant (20.5 *vs.* 12.8 months). Dalpiciclib was the first CDK4/6 inhibitor independently developed by Jiangsu Hengrui Medicin in China (3,4) which innovatively introduced a piperidine structure to lower liver toxicity. Results from DAWNA-2 clinical trial showed prolonged PFS of $HR^+/HER2^-$ ABC patients who received dalpiciclib + AI *vs.* placebo arm: 30.6 *vs.* 18.2 months. As for the safety profile, similar to previous CDK4/6 inhibitors, the most frequent adverse events of dalpiciclib are neutropenia and leukopenia (5). Trilaciclib is a first-in-class, short-acting CDK4/6 inhibitor. In animal studies, it was found to reduce the replication burden of bone marrow hematopoietic millipoietic cells through transient G1 blockade, thereby alleviating bone marrow hematopoietic stem cell depletion. The drug minimizes myelotoxicity while maintaining antitumor activity. In an open-label phase III trial in patients with metastatic triple negative breast cancer (TNBC), giving trilaciclib before gemcitabine plus carboplatin (GCb) significantly improved overall survival (OS) compared with GCb alone, possibly through the protection and direct activation of immune function (6). In a word, the five CDK4/6 inhibitors had similar efficacy and toxicity profiles in ABC treatment. Additionally, in the setting of early-stage breast cancer, the role of CDK4/6 inhibitors needs to be further identified. Only the monarchE trial, so far, supported abemaciclib in combination with endocrine therapy as an adjuvant treatment for $HR^+/HER2^-$ high risk early-stage breast cancer.

Although CDK4/6 inhibitors improved the condition of $HR^+/HER2^-$ ABC patients, most of them eventually developed acquired resistance. In this review analysis, various mechanisms and markers that may lead to resistance to CDK4/6 inhibitors were listed (Figure S1). Based on the current clinically approved CDK4/6 inhibitors, several possible methods to overcome drug resistance were also discussed here. This article is presented in accordance with the Narrative Review reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2807/rc).

Methods

A comprehensive, non-systematic review of the most recent literature was conducted to determine the use of CDK4/6 inhibitors in breast cancer research. Studies from 2015 to 2022 were reviewed from PubMed/MEDLINE using the keywords “Breast Cancer”, “Cyclin-dependent kinase4/6 (CDK4/6) inhibitor”, “resistant markers”. The last search was run on October 1, 2022. The language was limited to English. *Table 1* summarizes the search strategy. The literature search and systematic review identified 1,166 records. After intensive screening, we identified 452 eligible clinical studies. Through reading full texts, 50 studies were finally confirmed. Figure S1 is the specific process.

Resistance mechanisms of CDK4/6 inhibitors

CDK4/6 is a common downstream target of Ras/mitogen activated protein kinase (MAPK), estrogen receptor (ER), phosphoinositide 3 kinase (PI3K)/mechanistic target of rapamycin (mTOR), and other pro-growth signaling pathways (7). CDK4 and CDK6 can combine with three types of Cyclin D (Cyclin D1, Cyclin D2, and Cyclin D3). Correct synthesis and location of Cyclin D affect the activity of CDK4/6 (8). Generally, CDK4/6 combines with Cyclin D1, inducing retinoblastoma protein (Rb) phosphorylation and then Rb-E2F complex dissociation. Once E2F is released, it will activate genes required for DNA replication, promoting the cell cycle to enter the S phase from the G1 phase. This key regulatory mechanism of the cell cycle is

Table 1 The search strategy summary

Items	Specification
Date of search	October 1, 2022
Databases and other sources searched	PubMed/MEDLINE
Search terms used	Breast Cancer, Cyclin-dependent kinase4/6 (CDK4/6) inhibitor, resistant markers
Timeframe	January 2003 to September 2022
Inclusion and exclusion criteria	Clinical Trial; Meta-Analysis; Randomized Controlled Trial; Review; Systematic Review. Written in English language
Selection process	Consensus between co-authors

called the Cyclin D-CDK4/6-INK4-Rb pathway. Therefore, targeting CDK4/6 has attracted special interest as an anti-cancer treatment method (9).

CDK4/6 inhibitors, as small molecule inhibitors, target CDK4/6 ATP binding sites, which prevent CDK4/6 from forming a complex with Cyclin D. In this way, they can block the binding of ATP, thereby cutting off upstream growth signals and arresting the transition from G1 to S phase of the cell cycle (10,11). The key selected biomarkers with putative relevance in CDK4/6-targeted therapies are presented in *Table 2* (12-33).

Drug resistance due to abnormal Cyclin D-CDK4/6-Rb regulatory axis

Retinoblastoma gene (RB) mutation, Cyclin E-CDK2 axis activation, p16 amplification

CDK4/6 inhibitors act through the "Cyclin-CDK-Rb-E2F-cell cycle-related genes" axis. Preclinical studies have confirmed that Cyclin-CDK4/6 lacks downstream targets when the RB1 gene is deleted. In this case, the cell cycle can be promoted through bypass pathways, such as E2F activation and Cyclin-CDK2 regulatory axis, resulting in CDK4/6 inhibitors resistance. About 5% of CDK4/6 inhibitors resistant patients have these alterations (28,34). At the same time, E2F is released after RB1 gene inactivation, which induces the activation of Cyclin E to activate CDK1-3, further phosphorylating Rb to promote cell cycle progression.

P16, a member of the INK4 family, is a natural CDK4 inhibitor acting as an important tumor suppressor involved in the cell cycle regulation. Under natural conditions when functional Rb is present, p16 plays a role to suppress tumors, since CDK4/6 (a target of p16) requires Rb for its kinase activity. Lower levels of p16 can be observed in palbociclib-sensitive breast cancer cell lines (13), indicating that the overexpression of p16 may be related to the resistance of CDK4/6 inhibitors. However, this conclusion has not been confirmed clinically. It was found that low levels of p16 did not affect the prognosis of patients in a phase II clinical study of palbociclib (14). Although loss of Rb and overexpression of p16 seem to occur together, further studies are needed to reveal the connection between Rb and p16 in the mechanism.

CDK6 overexpression

CDK6 also plays an important role in the progression of the cell cycle from the G1 phase to the S phase in addition to CDK4. A recent study found that the low expression of CDK6 may be associated with high sensitivity to SHR6390 (35). CDK6 not only promotes the transition from the G1 phase to the S phase but also plays an important role in regulating the activity of transcription factors. The function of CDK6 mainly depends on the kinase, but there are also some kinase-independent functions. On the one hand, the possible mechanism of CDK6 amplification which causes resistance to CDK4/6 inhibitors may involve vascular endothelial growth factor A (VEGF-A) or p16. According to previous reports, CDK6 can up-regulate the transcription of p16 in the presence of signal transduction and activation factor 3 (STAT3) and Cyclin D. On the other hand, CDK6 up-regulates VEGF-A and C-Jun, which induces vascular tortuosity and usually promotes the progression of cancer and the generation of drug resistance (36).

Overexpression of CDK4

CDK4 is a component of the Cyclin D-CDK4/6-Rb pathway. CDK4 gene amplification, mutation, and epigenetic changes can activate this pathway, which may limit the efficacy of CDK4/6 inhibitors (16). However, the relationship between CDK4 overexpression and CDK4/6

Table 2 The mainly selected biomarkers with putative relevance in CDK4/6-targeted therapies

Biomarker	Clinical trial	Description	References
Cyclin-CDK4/6-Rb regulatory axis			
RB1 (RB2 and RBL1)	PALOMA-3	Main CDK4/6 substrates to repress cell-cycle-dependent transcription	Cen *et al.* 2012 (12) Finn *et al.* 2009 (13), Condorelli *et al.* 2018 (14)
CDK6	PALOMA-3	Direct target of CDK4/6 inhibitors	Yang *et al.* 2017 (15)
CDK4	PALOMA-3	Direct target of CDK4/6 inhibitors	Olanich *et al.* 2015 (16),
E2F		Downstream transcription factor of Rb	Chaussepied *et al.* 2004 (17)
HDACs	A phase I–II study of the histone deacetylase inhibitor vorinostat plus sequential weekly paclitaxel and doxorubicin-cyclophosphamide in locally advanced breast cancer	Remove acetyl from acetylated histones and inhibit p21	Zupkovitz *et al.* 2010 (18)
Other Cyclin and CDK-related drug resistance mechanisms			
MDM2	The EORTC-10994 randomized phase III trial	Negatively regulates the activity of p53	Laroche-Clary *et al.* 2017 (19)
CCNE1, CCNE2	MONALEESA-7	May drive CDK2/1 activation. Unclear kinase-independent functions	Franco *et al.* 2014 (20), Etemadmoghadam *et al.* 2013 (21)
TK1	Analysis of the randomised phase III EFECT	An S phase dependent enzyme of the cell cycle mainly exists in the cytoplasm	Bagegni *et al.* 2017 (22), Bonechi *et al.* 2019 (23)
AURKA	A phase II clinical trial of the Aurora and angiogenic kinase inhibitor ENMD-2076 for previously treated, advanced, or metastatic TNBC	A mitotic kinase that regulates ER expression. Synthetic lethal with RB1 loss	Willems E *et al.* 2018 (24)
CDK7		Regulate the transition from G2 phase to M phase	Schachter *et al.* 2013 (25)
CyclinD-CDK4/6-Rb bypass resistance mechanism			
FAT1		A tumor suppressor that represses the Hippo pathway	Li *et al.* 2018 (26)
Hippo pathway (LATS1, LAT2, and YAP)		The tumor suppressor Hippo pathway negatively regulates CDK6	Li *et al.* 2018 (26)
FGFR1/2	PALOMA-3	Aberrant FGFR signaling induces CCND1 expression and MAPK activation	Mao *et al.* 2020 (27), Wander *et al.* 2020 (28)
ER (AR)	Abemaciclib With Letrozole in Recurrent or Persistent Endometrial Cancer	ER (and AR) positivity suggests dependence on cyclinD-CDK4/6 complexes	Finn *et al.* 2009 (13), Wander *et al.* 2020 (28)
AP-1		A heterodimer composed of the nuclear protein transcription factors C-Fos and C-Jun	De Angelis *et al.* 2018 (29)
EMT pathway		The transformation of epithelial cells to mesenchymal cells	Lamouille *et al.* 2014 (30)

Table 2 (*continued*)

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Biomarker	Clinical trial	Description	References
Smad3		A component of the TGF-β signaling pathway	Yang *et al.* 2008 (31)
PTEN	Phase II trial of AKT inhibitor MK-2206 in patients with advanced breast cancer who have tumors with PIK3CA or AKT mutations, and/or PTEN loss/PTEN mutation	Loss of PTEN is associated with a poor prognosis in breast cancer	Costa *et al.* 2020 (32)
miR-432-5p		Mediates the transfer of resistance phenotype between adjacent cell populations	Andrikopoulou *et al.* 2021 (33)

CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; RB1/2, retinoblastoma 1/2; RBL1, retinoblastoma-like 1; HDACs, histone deacetylases; MDM2, mouse double minute 2; CCNE1, cyclin E1; CCNE2, cyclin E2; TK1, thymidine kinase 1; EFECT, Evaluation of Faslodex versus Exemestane Clinical Trial; AURKA, aurora kinase A; ER, estrogen receptor; CDK7, cyclin-dependent kinase 7; FAT1, fat atypical cadherin 1; LATS1, large tumor suppressor kinase 1; LAT2, linker for activation of T cells family member 2; YAP, yes-associated protein; FGFR1/2, fibroblast growth factor receptor 1/2; CCND1, recombinant cyclin D1; MAPK, mitogen activated protein kinase; AR, androgen receptor; AP-1, activator protein 1; EMT, epithelial mesenchymal transition; Smad3, smad family member3; TGF-β, transforming growth factor-β; PTEN, phosphatase and tensin homolog deleted on chromosome ten.

inhibitor resistance requires further research to determine whether this correlation is confined to a specific cancer type or a subtype.

Overexpression of E2F

E2F is the downstream transcription factor of Rb. Both Cyclin D-CDK4/6 complex and Cyclin E-CDK2 complex are able to induce Rb phosphorylation, releasing E2F and promoting the cell cycle into the S phase. Loss of Rb is related to the up-regulation of E2F expression, which leads to the structural activation of its downstream target protein. In addition, E2F can also up-regulate the protein kinase B (AKT) signal through Gab2 (17). The overexpression of E2F causes cancer cells to bypass the inhibition of CDK4/6 and to continue the cell cycle relying on other signaling pathways apart from the Cyclin D-CDK4/6 axis. It is suggested that the Cyclin D-CDK4/6-Rb axis is not an enclosed signaling regulatory pathway as many other bypass regulatory mechanisms can interact with E2F.

Overexpression of p16INK4 family genes

The p16INK4 family is a group of tumor suppressors that function to regulate CDK4/6 protein activity. p16INK4A (encoding CDKN2A), p15INK4B (encoding CDKN2B), p18INK4C (encoding CDKN2C) and p19INK4D (encoding CDKN2D) are members of this family. They inhibit Cyclin D-CDK4/6 complex formation through competitively binding to CDK and inducing conformational changes to inhibit ATP binding and cellular transition from G1 to S phase (37,38). Expression silencing of the CDKN2 gene family often occurs in tumor cells, and sensitivity to CDK4/6 inhibitors arises due to enhanced CDK4/6 activity resulting from the absence of upstream inhibitors. When these CDK4/6 repressors overexpress, the tumor cell cycle progression becomes partially independent of CDK4/6 signaling, resulting in resistance to CDK4/6 inhibitors.

Histone deacetylases (HDACs) activation

HDACs can remove acetyl from acetylated histones and inhibit p21 which interacts with Cyclin D throughout the cell cycle and interacts with Cyclin A or Cyclin B in the later periods of the cell cycle (G2/M phase) (39). HDACs activate p21 and cause cell cycle arrest in G1 and G2/M phases, enhancing the effect of CDK4/6 inhibition in drug-resistant cancer cells (40). It has been reported that HDACs and CDK4/6 inhibitors have a synergic effect on Noxa and Bim, unique proteins of apoptotic gene BH3, to induce the apoptosis of breast cancer cells (40). Therefore, HDACs can enhance the activity of CDK4/6 inhibitors through a variety of mechanisms.

Tucidinostat (formerly known as chidamide) is an oral subtype-selective HDAC inhibitor. In the ACE trial, the combination of tucidinostat with exemestane showed preliminary signs of encouraging anti-tumor activity in

patients with HR$^+$ ABC. It represented an important step forward in the development of epigenetic therapy for endocrine-resistant breast cancer and provided an important view for the potential of epigenetic targeting for overcoming anti-estrogen resistance. HDAC inhibitors could emerge as a new therapeutic tool in the rapidly evolving landscape of targeted therapies for this common disease (41).

Other Cyclin and CDK-related drug resistance mechanisms

Overexpression of CCNE1/2 and CDK2

It is found that the overexpression of CCNE1/2 (encoding Cyclin E) was closely related to CDK4/6 inhibitors resistance (20). Some studies suggested that CDK4/6 inhibitor resistant cancer cells no longer relied on the cyclin D1-CDK4/6 signaling pathway, but used alternative signaling pathways. For example, cancer cells may activate the Cyclin E-CDK2 pathway as a bypass. This suggests that the inhibition of Cyclin E-CDK2 can overcome the resistance of CDK4/6 inhibitors. It reported that cells that acquired resistance to CDK4/6 inhibitors due to CCNE1 amplification could be resensitized by targeting CDK2 (42).

CDK7 overexpression

CDK7 is a cell cycle regulator, which maintains the activity of CDK1 and CDK2 and regulates the transition from the G2 phase to the M phase. It also acts as a transcription factor binding with Cyclin H and MAT1 genes which participates in transcription regulation (17). According to reports, CDK7 has CAK activity to CDK4 and CDK6, which may play a role in G1 signal transduction (25).

THZ1 is a new selective and potent covalent CDK7 inhibitor, of which the IC50 (binding affinity) is 3.2 nM. The effect of THZ1 and/or tamoxifen have been evaluated in ER$^+$ breast cancer cell line MCF7 against tamoxifen resistant cell line LCC2 *in vitro* as well as in xenograft of breast cancer mouse models, revealing that THZ1 can enhance the cytotoxicity induced by tamoxifen and inhibit the expression of genes involved in tumor progression in MCF-7 and LCC2 cells. In *in vivo* experiments, THZ1 enhanced the effect of tamoxifen on tumor weight and tumor volume and decreased the expression of Ki67 and CD31. As a result, it increased the death rate of apoptotic cells. Therefore, CDK7 may be a therapeutic target for breast cancer.

Although CDK7 is involved in cell cycle regulation and transcription regulation, it is still unclear what is the specific mechanism of CDK7 in breast cancer and whether inhibiting the expression of CDK7 can overcome the resistance to CDK4/6 inhibitors.

MDM2 overexpression

MDM2 is a protein that negatively regulates the activity of p53. It has the effect of stabilizing and inhibiting cell senescence. 20–30% of breast cancer patients had a tumor with overexpression of MDM2. MDM2 blocks breast cancer cell apoptosis through a p53-dependent pathway, resulting in resistance to CDK4/6 inhibitors. The use of MDM2 inhibitors can reduce resistance to CDK4/6 inhibitors by inhibiting the activation of the MDM2-p53 complex. According to a previous research, the combined treatment of palbociclib with MDM2 inhibitor (RG7388) had a synergistic anti-cancer effect on human liposarcoma (19). In addition, the combination of another MDM2 inhibitor, CGM097, and CDK4/6 inhibitors and fulvestrant exhibited a considerable effect on cancer cells that were resistant to CDK4/6 inhibitors and endocrine therapy *in vivo* and *in vitro* (19).

TK1 activity

TK1, an S phase dependent enzyme, mainly exists in the cytoplasm. In a cell cycle, the level of TK1 begins to rise in the late G1 phase, reaches the peak in the S phase and begins to decline in the G2 phase. Due to the special correlation between the TK1 level and the S phase of the cell cycle, TK1 is also known as the "S phase key enzyme" relating to cell proliferation. Abnormally increased levels of TK1 can be caused by a sharp raise of DNA synthesis in uncontrollably proliferating cancer cells. After cell necrosis, the contents of cells are released into the blood, which results in a high level of TK1 in the serum. Thus, the detection of serum TK1 can be used in physical examinations, clinical screening of malignant proliferative lesions and the follow up after treatment.

TK1 presented prognostic value in HR$^+$ breast cancer patients. Recent data indicated that TK1 can be used as a marker of CDK4/6 inhibitors in patients who underwent neoadjuvant therapy [endocrine therapy (ET) + palbociclib] (22). TK1 expression and activity are regulated by palbociclib in HR$^+$ breast cancer cell lines, particularly in HER2$^+$ models. Ongoing studies of TKa in patients treated with palbociclib will assess the role of TKa as a circulating biomarker for predicting and monitoring response to CDK4/6 inhibitors (23). In the palbociclib-sensitive model, TK1 significantly reduced after 3 days of drug exposure compared with the control

group. At the same time, cell proliferation significantly decreased after at least 6 days. It indicated that TK1 may be an early marker for cell proliferation inhibition in cells which respond to palbociclib. Besides, studies have shown that patients with elevated TK1 levels had earlier disease progression compared with those with stable or decreased TK1 levels after treatment (43,44). In summary, progressive tumor proliferation indicated by elevated TK1 activity may also be a sign of early drug resistance.

CyclinD-CDK4/6-Rb bypass resistance mechanism

Major hormone and mitogenic pathways

Loss of FAT1

According to an analysis of 348 cases of ER$^+$ breast cancer treated with CDK4/6 inhibitors, it was determined that FAT1 and RB1 were related to loss of function mutations and CDK4/6 inhibitors resistance. Loss of FAT1 leads to a significant increase of CDK6 while inhibiting CDK6 can restore sensitivity to CDK4/6 inhibitors treatment. The induction of CDK6 is mediated by FAT1 through the Hippo pathway accompanied by the accumulation of YAP and TAZ transcription factors on the CDK6 promoter (26).

The PFS of patients with FAT1 mutation was 2.4 months, while the PFS of those without FAT1 mutation was 10.1 months. Studies have found that changing other components of the Hippo pathway could also promote resistance to CDK4/6 inhibitors (26,45). These findings revealed the suppressive effect of Hippo signaling in breast cancer and confirmed that loss of FAT1 was correlated to CDK4/6 inhibitors resistance.

Activation of fibroblast growth factor receptor (FGFR) pathway

FGFR signaling pathway participates significantly in biological processes, such as proliferation, differentiation, and cell survival. The FGFR pathway is frequently activated in many different types of cancers, including breast cancer. Studies have shown that FGFR1 amplification activated PI3K/AKT and RAS/MEK/extracellular signal-regulated kinase (ERK) signaling pathways in endocrine-resistant breast cancer cells (46,47). The FGFR pathway is mainly activated by FGF2 amplification (48). FGF2 mediates endocrine resistance, which can be enhanced by FGFR1 signaling (23), suggesting that downregulation of FGFR1 can prevent endocrine resistance (46). In addition, a recent study reported that overexpression of FGFR1 induced up-regulation of ER-mediated transcription, which may contribute to Palbociclib resistance (49). Therefore, combined inhibition of CDK4/6 and FGFR may be an option to overcome resistance to CDK4/6 inhibitors.

Activation of PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR signaling pathway is activated in 30–40% of breast cancers, especially in HR$^+$ breast cancer (50). It is a key factor in resistance to endocrine therapy. Previous studies confirmed that adding PI3K inhibitors to endocrine therapy could improve outcomes of ABC patients, especially for those with PIK3CA mutations (51). PI3K inhibitors can prevent the development of resistance to CDK4/6 inhibitors, but they are not able to re-sensitize cells which have acquired resistance (42).

It has been reported that inhibiting mTORC1/2 had no direct effect on ER function, but could reduce the expression of Cyclin D1, phosphorylated Rb and E2F. CDK4/6 inhibitors phosphorylate AKT through phosphoinositide dependent protein kinase 1, inducing activation of the PI3K/AKT pathway. Another recent study showed that PI3K inhibitors reduced the expression of Cyclin D1 and prevented the early adaptation to CDK4/6 inhibitors in ER$^+$ breast cancer. Thus, the combination of CDK4/6 inhibitors and PI3K inhibitors arose some interest. In a preclinical CDK4/6 inhibitors-sensitive model, CDK4/6 inhibitors plus PI3K inhibitors significantly improved the effect of tumor cell proliferation repression compared with monotherapy.

The activation of AKT is also related to the development of resistance to CDK4/6 inhibitors. It is found that part of the ribociclib resistant breast cancer cells appeared phosphatase and tensin homolog deleted on chromosome ten (PTEN) loss induced by AKT activation. Loss of PTEN was correlated with increased activation of CDK4 and CDK2, which eventually induced CDK4/6 inhibitors resistance (32).

Loss of ER or androgen receptor (AR) expression

The main driving factor of Cyclin-CDK4/6 activity in breast cancer cells is ER mediated by hormones. Losing the expression of ER or AR has been observed in preclinical models with abemaciclib resistance. ER and progesterone receptor (PR) expression loss before and after CDK4/6 inhibitors treatment was also detected by a paired biopsy in a small number of patients (15). These data indicated that the resistance to CDK4/6 inhibitors in some patients may be related to the decline of ER and PR levels.

AR is a steroid-hormone activated transcription factor belonging to the nuclear receptor superfamily. It has been reported that AR signaling pathway acted as a mediator of

the cell cycle and correlated with CDK4/6 resistance. AR binds to Cyclin D1 and regulates its expression in McF-7pr cells. AR activation increased the expression of CDK2 and CCNE1 in drug-resistant cell lines and AR blockade restored sensitivity to palbociclib (52). It suggested that targeting both AR and CKD4/6 may provide a new strategy for drug resistance.

Higher AP-1 transcriptional activity

AP-1 is a heterodimer composed of two nuclear protein transcription factors, C-Fos and C-Jun, regulating a variety of genes including CCND1. 20–40% of breast cancers have high levels of activating C-Jun, which interacts with and inhibits ER. It has been reported that either the overexpression of C-Jun or C-Fos could be the reason for resistance to CDK4/6 inhibitors and endocrine therapy. For example, the overexpression of C-Jun was found to be related to resistance to anti-estrogen therapy in the MCF-7 cell line. Higher AP-1 transcriptional activity and C-Fos levels were also determined to be associated with the resistance to palbociclib and tamoxifen (29). The mechanism of CDK4/6 inhibitors resistance by AP-1 overexpression may be related to the inhibitory effect of C-Jun on ER. In addition, the overexpression of Cyclin D1 transcribed by C-Jun may also be explained by this mechanism. Therefore, the down-regulation of C-Jun may be able to improve the condition of CDK4/6 inhibitors resistance. The synergistic effect of Palbociclib and AP-1 blocking has been observed to be able to inhibit the proliferation of breast cancer cells, especially when accompanied by fulvestrant (29). Currently, one selective c-Fos/AP-1 inhibitor (T-5224) has progressed to phase II clinical trials. The combined use of this AP-1 inhibitor and CDK4/6 inhibitors may have a certain effect in reducing the acquired resistance to CDK4/6 inhibitors.

Epithelial mesenchymal transition (EMT) pathway

The transformation of epithelial cells to mesenchymal cells is called EMT, which promotes tumor invasion and metastasis (53). It also participates in the formation of resistance to anti-tumor drugs, including CDK4/6 inhibitors. According to reports, CDK4/6 inhibitors the occurrence of EMT through the activation of TGF-β. Phosphorylated TGF-β activates Smad2 and Smad3 and forms complexes with Smad4, leading to the activation of EMT (30). Phosphorylated TGF-β also induces EMT/mTOR signals through PI3K/AKT. Therefore, TGF-β or EMT inhibition is also a potential option for overcoming resistance to CDK4/6 inhibitors.

Smad3 inhibition

Smad3, a component of the TGF-β signaling pathway, can cause G1 phase arrest. It is reported that Smad3 was able to restore the cell cycle of breast cancer cells by inhibiting Rb-E2F, as a downstream factor of phosphorylated Cycline E-CDK2 complex and Cycline D1-CDK4/6 complex (31). In an anti-trastuzumab model, the Smad3 structure that inhibits the mutation of the CDK2 phosphorylation site was transfected into Trastuzumab resistant breast cancer cells, resulting in the reduction of the phosphorylation level of Smad3 and cancer proliferation.

FOXM1 overexpression

FOXM1 is a key regulator of the cell cycle. Overexpression of FOXM1 relates to tumor invasion, drug resistance, and poor prognosis. There has been a study showing that dalpiciclib and pyrotinib could reduce the phosphorylation of the FOXM1 and inhibit breast cancer cell proliferation (54). There was also evidence that FOXM1 knockout could restore the sensitivity to endocrine therapy (55).

Amplification of AURKA

AURKA is a mitotic kinase that regulates ER expression. AURKA amplification is enriched in CDK4/6 inhibitors resistant ER^+ breast cancer cells (28), suggesting its potential predictive value for drug resistance. AURKA inhibition and RB1 loss revealed an effect of synthetic lethality on cancer cells. In a preclinical study, the application of an AURKA inhibitor in cancer cells with RB1 mutation promoted a durable regression of RB1 mutation (56).

Other cell cycle-nonspecific mechanisms

Autophagy inhibition

Autophagy is considered to be a mechanism of tumor cell tolerance and survival (57). Autophagy can be activated in the condition of cell cycle arrest, mediating the arrest of the G1 phase and the reversal of aging (57). In preclinical studies, inhibition of autophagy was able to enhance the effectiveness of many kinds of anticancer drugs (58,59). There was also some evidence about the correlation between autophagy and CDK4/6 inhibitors resistance. CDK4/6 inhibitors can activate autophagy by inhibiting Cyclin D1, thereby inhibiting the proliferation of breast epithelial cells (57).

Cell senescence

CDK4/6 inhibitors induce cell senescence by cell cycle blockade, which makes cells present morphological characteristics of senescence, including cell enlargement, an increase of β-galactosidase activity and formation of senescence-associated heterochromatin foci (SAHF) (60). Additionally, CDK4/6 can activate FOXM1 to maintain the expression of G1/S cycle-related genes and prevent

cancer cells from senescence (61). Research showed that dalpiciclib induced G1 phase cell cycle arrest and cellular senescence (35).

Alterations in tumor immune microenvironment

Recently, with the arising focus on the cancer immune microenvironment, its relationship with cancer drug resistance gradually emerges. In a preclinical study, CDK4/6 inhibitors were observed to promote anti-tumor immunity similar to other targeted therapies. CDK4/6 inhibitors could reduce the activity of DNA methyltransferase, enhance NFAT activity as well as elevate the level of inflammatory cytokines. These produced an unfavorable inflammatory tumor microenvironment for CDK4/6 inhibitors (62).

There are still more resistance markers under studying. A study indicates that patients with high levels of polo-like kinase 1 (PLK-1) gene expression, treated with palbociclib in the phase III PEARL study, have worse mPFS compared to patients with low PLK-1 pretreatment tumors. Early data suggested that insulin-like growth factor 1 receptor amplification may be associated with resistance to CDK4/6 inhibition (63).

Clinical trials of CDK4/6 inhibitors

There are many clinical trials on CDK4/6 inhibitors (*Table 3*). These clinical trials provide various directions to further understand the indications of CDK4/6 inhibitors and help find innovative treatment strategies for CDK4/6 inhibitors resistance.

In $HR^+/HER2^-$ breast cancer, DAWNA-1 (NCT03927456), a double-blind, randomized, phase 3 trial of dalpiciclib + fulvestrant for HR^+, $HER2^-$ ABC with disease progression after endocrine therapy. Patients were randomly assigned 2:1 to receive dalpiciclib + fulvestrant or placebo + fulvestrant. The study met its primary endpoint, showing significantly longer investigator-assessed PFS with dalpiciclib + fulvestrant compared with placebo + fulvestrant (median =15.7 *vs.* 7.2 months). The most common grade 3 or 4 adverse events with dalpiciclib + fulvestrant were neutropenia (84.2%) and leukopenia (62.1%). Its clinical efficacy is similar to other CDK4/6 inhibitors.

Professor Matthew P. Goetz reported median OS results from the second interim analysis of the MONARCH-3 study, showing that for first-line treatment of $HR^+/HER2^-$ ABC, the NSAI + abemaciclib extended median OS from 54.5 to 67.1 months compared to NSAI + placebo. OS data are not yet mature and the final OS analysis will be performed in 2023 (64).

In $HR^+/HER2^+$ breast cancer, the application of CDK4/6 inhibitors has also achieved progress. The LORDSHIPS study is a single-center phase Ib/II clinical trial which is the first all-oral chemotherapy-free study (dalpiciclib + pyrrolizidine + letrozole) in the front-line $HR^+/HER2^+$ metastatic breast cancer setting. The median PFS was 11.3 months with an objective remission rate of 66.7%. The median PFS in the first line has not been reached and the median PFS in the second line was 10.9 months. In the 2022 European Society for Medical Oncology (ESMO), the results of the previous monarcHER study showed that abemaciclib + trastuzumab + fulvestrant significantly improved median PFS compared with trastuzumab + chemotherapy in patients with $HR^+/HER2^+$ ABC (8.3 *vs.* 5.7 months) (65). The current updated data showed that abemaciclib + trastuzumab + fulvestrant prolonged median OS by nearly 1 year compared with trastuzumab + chemotherapy with a good safety profile (31.1 *vs.* 20.7 months), consistent with previous studies (66).

PADA-1 is a multicenter, phase 3, randomized controlled study that investigated the feasibility of large-scale real-time serial monitoring of drug resistance-associated mutations by circulating tumor DNA analysis in patients with $ER^+/HER2^-$ ABC during the first-line treatment with aromatase inhibitors in combination with palbociclib. The results demonstrate for the first time that switching to endocrine therapy in patients with elevated blood ESR1 mutation levels significantly doubles PFS without an increase in toxic effects. Therefore, early switching after the occurrence of ESR1 mutations in the blood may provide significant clinical benefit (67).

The Phase II RIGHT Choice study presented at San Antonio Breast Cancer Symposium (SABCS) 2022 gives us another perspective on the status of CDK4/6 inhibitors. The study focuses on $HR^+/HER2^-$ ABC patients with highly aggressive disease. In some guidelines and consensus, chemotherapy or combination chemotherapy is recommended for this group of patients. This study evaluated the efficacy of CDK4/6 inhibitors + endocrine therapy *vs.* the investigator's choice of chemotherapy in $HR^+/HER2^-$ ABC and showed that CDK4/6 inhibitors + endocrine therapy significantly improved PFS *vs.* chemotherapy. Thus, this study again tells us that CDK4/6 inhibitors + endocrine therapy is a reliable option even for patients with more aggressive $HR^+/HER2^-$ ABC (68).

There are also attempts to apply CDK4/6 inhibitors in other subtypes of breast cancer. A study showed the synergistic effect of CDK4/6 inhibitors and PI3 kinase

Table 3 CDK4/6 inhibitors in active clinical trials

Drug name	Study title	Developer	Interventions	Targets (IC50)	Conditions	NCT identifier	First posted
$HR^+/HER2^-$							
Palbociclib, PD-0332991, Ibrance	A Study of Palbociclib (PD-0332991) + Letrozole *vs.* Letrozole For 1st Line Treatment Of Postmenopausal Women With ER+/HER2– Advanced Breast Cancer (PALOMA-2)	Pfizer	Drug: PD-0332991; drug: letrozole; drug: placebo	CDK4 (11 nM); CDK6 (16 nM)	Breast neoplasms	NCT01740427	Dec 4, 2012
	Palbociclib (PD-0332991) Combined With Fulvestrant In Hormone Receptor+ HER2-Negative Metastatic Breast Cancer After Endocrine Failure (PALOMA-3)	Pfizer	Drug: palbociclib; drug: fulvestrant; drug: placebo		Metastatic breast cancer	NCT01942135	Sep 13, 2013
Ribociclib, LEE-011	Study of Efficacy and Safety of LEE011 in Postmenopausal Women With Advanced Breast Cancer (MONALEESA-2)	Novartis International	Drug: LEE011; drug: letrozole; drug: LEE011 placebo	CDK4 (10 nM); CDK6 (39 nM)	Advanced metastatic breast cancer	NCT01958021	Oct 8, 2013
	Study of Efficacy and Safety of LEE011 in Men and Postmenopausal Women With Advanced Breast Cancer (Monaleesa3)	Novartis International	Drug: ribociclib; drug: fulvestrant; drug: ribociclib placebo		Advanced breast cancer	NCT02422615	Apr 21, 2015
	Study of Efficacy and Safety in Premenopausal Women With Hormone Receptor Positive, HER2-negative Advanced Breast Cancer (Monaleesa7)	Novartis International	Drug: LEE011; drug: tamoxifen; drug: letrozole (and 3 more...)		Advanced metastatic breast cancer	NCT02278120	Oct 29, 2014
Abemaciclib, LY2835219, Verzenios	A Study of Abemaciclib (LY2835219) In Participants With Previously Treated Breast Cancer That Has Spread (MONARCH 1)	Eli Lilly	Drug: abemaciclib	CDK4 (12 nM); CDK6 (10 nM); CDK9 (57 nM)	Metastatic breast cancer	NCT02102490	Apr 3, 2014
	A Study of Abemaciclib (LY2835219) Plus Tamoxifen or Abemaciclib Alone in Women With Metastatic Breast Cancer (Next MONARCH 1)	Eli Lilly	Drug: abemaciclib; drug: tamoxifen; drug: prophylactic loperamide		Metastatic breast cancer	NCT02747004	Apr 21, 2016
	A Study of Abemaciclib (LY2835219) Combined With Fulvestrant in Women With Hormone Receptor Positive HER2 Negative Breast Cancer Monarch2 (MONARCH 2)	Eli Lilly	Drug: abemaciclib; drug: fulvestrant; drug: placebo		Breast neoplasms	NCT02107703	Apr 8, 2014
	MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer	Eli Lilly	Drug: abemaciclib; drug: letrozole or anastrozole; drug: placebo		Metastatic breast cancer	NCT02246621	Nov 10, 2017

Table 3 (*continued*)

Table 3 (*continued*)

Drug name	Study title	Developer	Interventions	Targets (IC50)	Conditions	NCT identifier	First posted
SHR6390	A Study of SHR6390 in Combination With Letrozole or Anastrozole in Patients With HR Positive and HER2 Negative Advanced Breast Cancer	Jiangsu Hengrui Medicine	Drug: SHR6390; drug: placebo tablets; drug: letrozole or anastrozole tablets	CDK4 (12 nM); CDK6 (10 nM)	Advanced breast cancer	NCT03966898	May 29, 2019
	A phase 1 study of dalpiciclib, a cyclin-dependent kinase 4/6 inhibitor in Chinese patients with advanced breast cancer	Jiangsu Hengrui Medicine	Drug: SHR6390		Advanced breast cancer	NCT02684266	Nov 4, 2021
Dalpiciclib (SHR6390)	Dalpiciclib or placebo plus Fulvestrant in hormone receptor-positive and HER2-negative advanced breast cancer: a randomized, phase 3 trial	Jiangsu Hengrui Medicine	Drug: SHR6390; drug: placebo	CDK4 (12.4 nM); CDK6 (9.9 nM)	Advanced breast cancer	NCT03927456	Apr 12, 2021
Lerociclib, G1T38	G1T38, a CDK 4/6 Inhibitor, in Combination With Fulvestrant in Hormone Receptor-Positive, HER2-Negative Locally Advanced or Metastatic Breast Cancer	G1 Therapeutics	Drug: G1T38; drug: fulvestrant	CDK4 (1 nM); CDK6 (2 nM); CDK9 (28 nM)	Carcinoma, ductal, breast cancer, breast neoplasm	NCT02983071	Dec 6, 2016
PF-06873600	A Safety, Pharmacokinetic, Pharmacodynamic and Anti-Tumor Study of PF-06873600 as a Single Agent and in Combination With Endocrine Therapy	Pfizer	Drug: PF-06873600; drug: endocrine therapy 1; drug: endocrine therapy 2	CDK2 (0.09 nM Ki); CDK4 (0.13 nM Ki); CDK6 (0.16 nM Ki)	HR^+ $HER2^-$ metastatic breast cancer, ovarian cancer, fallopian tube cancer, primary peritoneal cancer, TNBC, male breast cancer	NCT03519178	May 8, 2018
FCN-437c	CDK4/6 inhibitor FCN-437c in combination with letrozole for HR+/HER2-advanced breast cancer	Fochon Pharmaceuticals	Drug: FCN-437	CDK4; CDK6	Solid tumor, adult	NCT03951116	May 15, 2019
BPI-16350	A Phase I Study of BPI-16350 in Patients With Advanced Solid Tumor	Betta Pharmaceuticals	Drug: BPI-16350	CDK4; CDK6	Advanced solid cancer	NCT03791112	Jan 2, 2019
XZP-3287, Birociclib	Clinical study of XZP-3287 in the treatment of advanced malignant solid tumor stage Ia	Jilin Sihuan Pharmaceutical/ XuanZhu Pharma	Drug: birociclib	CDK4; CDK6	Phase I for advanced solid tumors	CTR20180020	Feb 9, 2018
HS-10342	A Phase 1 Study of HS-10342 In Patients With Advanced Solid Tumor	Jiangsu Hansoh Pharmaceutical Group	Drug: HS-10342	CDK4; CDK6	Advanced solid tumor, drug: HS-10342 Phase 1	NCT04060511	Aug 19, 2019
CS3002	A Study of CS3002 in Subjects With Advanced Solid Tumors	CStone Pharmaceuticals	Drug: CS3002	CDK4; CDK6	Advanced solid tumor	NCT04162301	Nov 14, 2019

Table 3 (*continued*)

Table 3 (*continued*)

Drug name	Study title	Developer	Interventions	Targets (IC50)	Conditions	NCT identifier	First posted
$HR^+/HER2^+$							
Abemaciclib, LY2835219, Verzenios	Final OS for abemaciclib plus trastuzumab +/− fulvestrant versus trastuzumab plus chemotherapy in patients with HR+, HER2+ advanced breast cancer (monarcHER): A randomized, open-label, phase II trial	Eli Lilly	Drug: abemaciclib; drug: trastuzumab; drug: fulvestrant	CDK4 (2 nM); CDK6 (10 nM); CDK9 (57 nM)	Metastatic breast cancer	NCT02675231	Jun 21, 2020
SHR6390	Study to Evaluate the Efficacy and Safety of CDK4/6 Inhibitor SHR6390 Combined With Pyrotinib in the Treatment of HER2-positive Advanced Breast Cancer	Jiangsu Hengrui Medicine	Drug: pyrotinib combine with SHR6390	CDK4 (12 nM); CDK6 (10 nM)	Metastatic breast cancer	NCT03993964	Jun 21, 2019
Dalpiciclib (SHR6390)	Dalpiciclib Combined With Pyrotinib and Letrozole in Women With HER2-Positive, Hormone Receptor-Positive Metastatic Breast Cancer (LORDSHIPS): A Phase Ib Study		Drug: dalpiciclib; drug: pyrotinib; drug: letrozole	CDK4 (12.4 nM); CDK6 (9.9 nM)	Metastatic breast cancer	NCT03772353	Mar 7, 2022
TNBC							
Trilaciclib, G1T28	Trilaciclib (G1T28), a CDK 4/6 Inhibitor, in Combination With Gemcitabine and Carboplatin in Metastatic TNBC	G1 Therapeutics	Drug: trilaciclib; drug: gemcitabine; drug: carboplatin	CDK4 (1 nM); CDK6 (4 nM); CDK9 (50 nM)	Triple-negative breast neoplasms, breast neoplasm, breast cancer, TNBC	NCT02978716	Dec 1, 2016

CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; NCT, national clinical trial; OS, overall survival; TNBC, triple negative breast cancer.

inhibitors in PIK3CA-mutant TNBC cell lines and cell-cycle dynamics were observed to determine the response to CDK4/6 inhibitors, suggesting that some TNBC cells may be sensitive to this combined therapy (69).

Currently, endocrine therapy is recommended in preference for HR^+ ABC patients with visceral metastases, while chemotherapy is recommended in preference for domestic and international guidelines for the visceral crisis. With the intensification of endocrine combination therapy and the availability of CDK4/6 inhibitors in recent years, real-world cases of HR^+ breast cancer patients with visceral crisis treated with CDK4/6 inhibitors in combination with endocrine therapy have been reported. CDK4/6 inhibitors will continue to expand indications.

The way to solve CDK4/6 inhibitors resistance

CDK4/6 inhibitors have become the standard first-line treatment recommendation for $HR^+/HER2^-$ ABC. The PALOMA series of clinical trials with palbociclib as the primary investigational agent, the MONARCH series led by abemaciclib and the MONALESSA series led by ribociclib all showed that first-line and second-line use of CDK4/6 inhibitors in combination with endocrine therapy significantly prolonged PFS by nearly twofold compared with single endocrine therapy However, it is unknown whether different ER, PR and HER2 expression levels would lead to varying degrees of response to CDK4/6 inhibitors. PFS in patients with high ctDNA scores was poor with either palbociclib + fulvestrant or placebo + fulvestrant. A group of high-risk patients identified by ctDNA prior to treatment had poor clinical outcomes despite the addition of palbociclib. This suggests that multiple approaches need to be performed simultaneously to predict the sensitivity of patients to CDK4/6 inhibitors (70). Moreover, drug resistance is still an inevitable problem. Therefore, options for second-line and later-line treatment still need to be explored.

CDK4/6 inhibitors crossover therapy

There are currently several relevant clinical studies undergoing internationally, such as cross-line treatment with CDK4/6 inhibitors. The MAINTAIN trial was a randomized trial on patients with $HR^+/HER2^-$ breast cancer that progressed during any CDK4/6 inhibitors or endocrine treatment. Patients were randomized to either endocrine therapy + ribociclib (trial group) or endocrine therapy + placebo (control group). The trial group patients showed a statistically significant improvement in PFS *vs.* the placebo group (5.29 *vs.* 2.76 months), confirming that patients could still benefit from CDK4/6 inhibitors after a former failure of CDK4/6 inhibitors treatment (*Figure 1*) (71). However, patients' response to single agent hormone therapy after CDK4/6 inhibitors was limited, which has been evidenced in the EMERALD study and the VERONICA study (72,73).

Using endocrine or targeted therapies

Tucidinostat (formerly known as chidamide) is an HDAC inhibitor. Based on the results of the ACE study, tucidinostat was approved for the treatment of postmenopausal $HR^+/HER2^-$ ABC patients who have relapsed or progressed disease after endocrine therapy (41). At present, tucidinostat has shown promising results in real-world studies enrolling patients who have failed CDK4/6 inhibitors therapy.

Alpelisib is an oral selective PI3K inhibitor. The SOLAR-1 study explored the efficacy of alpelisib + fulvestrant in $HR^+/HER2^-$ men or postmenopausal women with ABC. The results showed a significant improvement in median PFS from 5.7 to 11.0 months in the alpelisib + fulvestrant group compared to placebo + fulvestrant in patients with PI3K mutations (51). The BYLieve study included a cohort of men or women with $HR^+/HER2^-$ ABC with PI3KCA mutations who had progressed on CDK4/6 inhibitors. Patients were given alpelsisib + endocrine therapy after CDK4/6 inhibitors. LT (long time) and VLT (very long time) disease control were observed in 25.6% and 16.5% of patients, respectively. Visceral disease, adverse effect occurrence, and ESR1 mutations did not prevent LT/VLT disease control. These data confirm that targeting PIK3CA mutations with alpelisib + fulvestrant after CDK4/6 inhibitors treatment may lead to LT disease control (74). A portion of retrospective studies and real-world studies suggest that mTOR inhibitors are also a treatment option for patients who progress on CDK4/6 inhibitors (75).

Using new antibody drug conjugate (ADC) drugs

ADCs have also been explored in patients progressing on CDK4/6 inhibitors. The TROPiCS-02 study suggests that sacituzumab govitecan has some value in this group of patients (76). DESTINY-Breast-04 study also enrolled a subset of patients resistant to CDK4/6 inhibitors and aimed to explore the efficacy of T-DXd in HR^+/HER2 low-

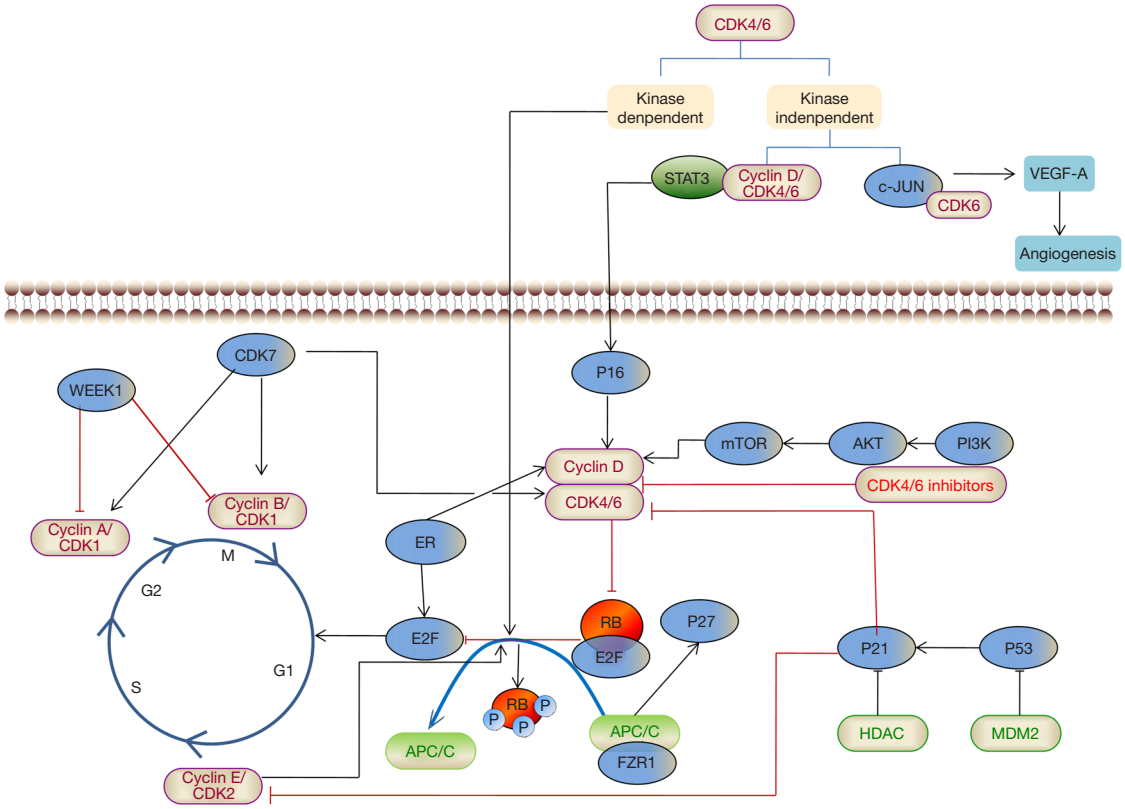

Figure 1 Resistance mechanism of CDK4/6 inhibitors. CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; STAT3, signal transduction and activation factor 3; VEGF-A, vascular endothelial growth factor A; CDK7, cyclin-dependent kinase 7; mTOR, mechanistic target of rapamycin; AKT, protein kinase B; PI3K, phosphoinositide 3 kinase; ER, estrogen receptor; RB, retinoblastoma; HDAC, histone deacetylase; MDM2, mouse double minute 2.

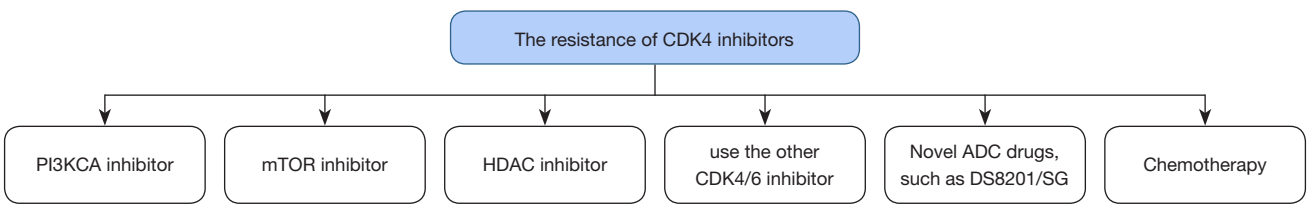

Figure 2 The application of inhibitors against the resistance of CDK4/6 inhibitors. CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; PI3KCA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; mTOR, mechanistic target of rapamycin; HDAC, histone deacetylase; ADC, antibody drug conjugate.

expressing ABCs patients.

Currently, no standard treatment regimen is recommended for patients after failure of first-line treatment with CDK4/6 inhibitors in guidelines, because the aforementioned regimens have not been compared head-to-head (*Figure 2*). The choice of a treatment regimen for this group of patients is currently based on their economic conditions, willingness to treatment, the presence of specific targets, and the accessibility of drugs to select the appropriate treatment modality.

Conclusions

The rise of CDK4/6 inhibitors has brought breakthroughs in the precision treatment of $HR^+/HER2^-$ ABC, which

greatly prolonged the survival of patients and improved their quality of life. In the post-CDK4/6 inhibitors era, further advances in basic medicine and translational research are needed to explore the resistance mechanisms of CDK4/6 inhibitors and develop corresponding treatment strategies. The trend of individualized treatment based on precisive biomarker detection has great potential for overcoming resistance to CDK4/6 inhibitors and making rational use of it.

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Footnote

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Hart CD, Migliaccio I, Malorni L, et al. Challenges in the management of advanced, ER-positive, HER2-negative breast cancer. Nat Rev Clin Oncol 2015;12:541-52.
3. Sheikh MS, Satti SA. The emerging CDK4/6 inhibitor for breast cancer treatment. Mol Cell Pharmacol 2021;13:9-12.
4. Zhang P, Xu B, Gui L, et al. A phase 1 study of dalpiciclib, a cyclin-dependent kinase 4/6 inhibitor in Chinese patients with advanced breast cancer. Biomark Res 2021;9:24.
5. Xu B, Zhang QY, Zhang P, et al. LBA16 Dalpiciclib plus letrozole or anastrozole as first-line treatment for HR+/HER2-advanced breast cancer (DAWNA-2): A phase III trial. Ann Oncol 2022;33:S1384-5.
6. Goel S, Tan AR, Rugo HS, et al. Trilaciclib prior to gemcitabine plus carboplatin for metastatic triple-negative breast cancer: phase III PRESERVE 2. Future Oncol 2022;18:3701-11.
7. Rocca A, Farolfi A, Bravaccini S, et al. Palbociclib (PD 0332991) : targeting the cell cycle machinery in breast cancer. Expert Opin Pharmacother 2014;15:407-20.
8. Ingham M, Schwartz GK. Cell-Cycle Therapeutics Come of Age. J Clin Oncol 2017;35:2949-59.
9. Goetz MP, Toi M, Campone M, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol 2017;35:3638-46.
10. Dukelow T, Kishan D, Khasraw M, et al. CDK4/6 inhibitors in breast cancer. Anticancer Drugs 2015;26:797-806.
11. Schoninger SF, Blain SW. The Ongoing Search for Biomarkers of CDK4/6 Inhibitor Responsiveness in Breast Cancer. Mol Cancer Ther 2020;19:3-12.
12. Cen L, Carlson BL, Schroeder MA, et al. p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells. Neuro Oncol 2012;14:870-81.
13. Finn RS, Dering J, Conklin D, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 2009;11:R77.
14. Condorelli R, Spring L, O'Shaughnessy J, et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol 2018;29:640-5.
15. Yang C, Li Z, Bhatt T, et al. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene

2017;36:2255-64.
16. Olanich ME, Sun W, Hewitt SM, et al. CDK4 Amplification Reduces Sensitivity to CDK4/6 Inhibition in Fusion-Positive Rhabdomyosarcoma. Clin Cancer Res 2015;21:4947-59.
17. Chaussepied M, Ginsberg D. Transcriptional regulation of AKT activation by E2F. Mol Cell 2004;16:831-7.
18. Zupkovitz G, Grausenburger R, Brunmeir R, et al. The cyclin-dependent kinase inhibitor p21 is a crucial target for histone deacetylase 1 as a regulator of cellular proliferation. Mol Cell Biol 2010;30:1171-81.
19. Laroche-Clary A, Chaire V, Algeo MP, et al. Combined targeting of MDM2 and CDK4 is synergistic in dedifferentiated liposarcomas. J Hematol Oncol 2017;10:123.
20. Franco J, Witkiewicz AK, Knudsen ES. CDK4/6 inhibitors have potent activity in combination with pathway selective therapeutic agents in models of pancreatic cancer. Oncotarget 2014;5:6512-25.
21. Etemadmoghadam D, Weir BA, Au-Yeung G, et al. Synthetic lethality between CCNE1 amplification and loss of BRCA1. Proc Natl Acad Sci U S A 2013;110:19489-94.
22. Bagegni N, Thomas S, Liu N, et al. Serum thymidine kinase 1 activity as a pharmacodynamic marker of cyclin-dependent kinase 4/6 inhibition in patients with early-stage breast cancer receiving neoadjuvant palbociclib. Breast Cancer Res 2017;19:123.
23. Bonechi M, Migliaccio I, Benelli M, et al. Abstract P6-09-02: Effects of palbociclib on thymidine kinase-1 (TK1) in hormone receptor positive (HR+) breast cancer cell lines. Cancer Res 2019;79:P6-09-02.
24. Willems E, Dedobbeleer M, Digregorio M, et al. The functional diversity of Aurora kinases: a comprehensive review. Cell Div 2018;13:7.
25. Schachter MM, Merrick KA, Larochelle S, et al. A Cdk7-Cdk4 T-loop phosphorylation cascade promotes G1 progression. Mol Cell 2013;50:250-60.
26. Li Z, Razavi P, Li Q, et al. Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. Cancer Cell 2018;34:893-905.e8.
27. Mao P, Cohen O, Kowalski KJ, et al. Acquired FGFR and FGF Alterations Confer Resistance to Estrogen Receptor (ER) Targeted Therapy in ER(+) Metastatic Breast Cancer. Clin Cancer Res 2020;26:5974-89.
28. Wander SA, Cohen O, Gong X, et al. The Genomic Landscape of Intrinsic and Acquired Resistance to Cyclin-Dependent Kinase 4/6 Inhibitors in Patients with Hormone Receptor-Positive Metastatic Breast Cancer. Cancer Discov 2020;10:1174-93.
29. De Angelis C, Nardone A, Cataldo ML, et al. Abstract P4-03-05: AP-1 as a potential mediator of resistance to the cyclin-dependent kinase (CDK) 4/6-inhibitor palbociclib in ER-positive endocrine-resistant breast cancer. Cancer Res 2018;78:P4-03-05.
30. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15:178-96.
31. Yang J, Song K, Krebs TL, et al. Rb/E2F4 and Smad2/3 link survivin to TGF-beta-induced apoptosis and tumor progression. Oncogene 2008;27:5326-38.
32. Costa C, Wang Y, Ly A, et al. PTEN Loss Mediates Clinical Cross-Resistance to CDK4/6 and PI3Kα Inhibitors in Breast Cancer. Cancer Discov 2020;10:72-85.
33. Andrikopoulou A, Shalit A, Zografos E, et al. MicroRNAs as Potential Predictors of Response to CDK4/6 Inhibitor Treatment. Cancers (Basel) 2021;13:4114.
34. Dean JL, Thangavel C, McClendon AK, et al. Therapeutic CDK4/6 inhibition in breast cancer: key mechanisms of response and failure. Oncogene 2010;29:4018-32.
35. Wang J, Li Q, Yuan J, et al. CDK4/6 inhibitor-SHR6390 exerts potent antitumor activity in esophageal squamous cell carcinoma by inhibiting phosphorylated Rb and inducing G1 cell cycle arrest. J Transl Med 2017;15:127.
36. Gacche RN, Assaraf YG. Redundant angiogenic signaling and tumor drug resistance. Drug Resist Updat 2018;36:47-76.
37. Baker SJ, Reddy EP. CDK4: A Key Player in the Cell Cycle, Development, and Cancer. Genes Cancer 2012;3:658-69.
38. LaPak KM, Burd CE. The molecular balancing act of p16(INK4a) in cancer and aging. Mol Cancer Res 2014;12:167-83.
39. Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. J Clin Oncol 2009;27:5459-68.
40. Lee J, Lim B, Pearson T, et al. Abstract P5-21-15: The synergistic antitumor activity of entinostat (MS-275) in combination with palbociclib (PD 0332991) in estrogen receptor-positive and triple-negative breast cancer. Cancer Res 2018;78:P5-21-15.
41. Jiang Z, Li W, Hu X, et al. Tucidinostat plus exemestane for postmenopausal patients with advanced, hormone receptor-positive breast cancer (ACE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2019;20:806-15.
42. Herrera-Abreu MT, Palafox M, Asghar U, et al. Early Adaptation and Acquired Resistance to CDK4/6 Inhibition

in Estrogen Receptor-Positive Breast Cancer. Cancer Res 2016;76:2301-13.
43. Bitter EE, Townsend MH, Erickson R, et al. Thymidine kinase 1 through the ages: a comprehensive review. Cell Biosci 2020;10:138.
44. McCartney A, Bonechi M, De Luca F, et al. Plasma Thymidine Kinase Activity as a Biomarker in Patients with Luminal Metastatic Breast Cancer Treated with Palbociclib within the TREnd Trial. Clin Cancer Res 2020;26:2131-9.
45. Li Z, Zou W, Zhang J, et al. Mechanisms of CDK4/6 Inhibitor Resistance in Luminal Breast Cancer. Front Pharmacol 2020;11:580251.
46. Turner N, Pearson A, Sharpe R, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 2010;70:2085-94.
47. Sobhani N, Fassl A, Mondani G, et al. Targeting Aberrant FGFR Signaling to Overcome CDK4/6 Inhibitor Resistance in Breast Cancer. Cells 2021;10:293.
48. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116-29.
49. Cheng Q, Ma Z, Shi Y, et al. FGFR1 Overexpression Induces Cancer Cell Stemness and Enhanced Akt/Erk-ER Signaling to Promote Palbociclib Resistance in Luminal A Breast Cancer Cells. Cells 2021;10:3008.
50. Takeshita T, Yamamoto Y, Yamamoto-Ibusuki M, et al. Clinical significance of plasma cell-free DNA mutations in PIK3CA, AKT1, and ESR1 gene according to treatment lines in ER-positive breast cancer. Mol Cancer 2018;17:67.
51. André F, Ciruelos EM, Juric D, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol 2021;32:208-17.
52. Ji W, Shi Y, Wang X, et al. Combined Androgen receptor blockade overcomes the resistance of breast cancer cells to palbociclib. Int J Biol Sci 2019;15:522-32.
53. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010;29:4741-51.
54. Wang Y, Yuan X, Li J, et al. The Synergistic Effects of SHR6390 Combined With Pyrotinib on HER2+/HR+ Breast Cancer. Front Cell Dev Biol 2021;9:785796.
55. Bergamaschi A, Madak-Erdogan Z, Kim YJ, et al. The forkhead transcription factor FOXM1 promotes endocrine resistance and invasiveness in estrogen receptor-positive breast cancer by expansion of stem-like cancer cells. Breast Cancer Res 2014;16:436.
56. Gong X, Du J, Parsons SH, et al. Aurora A Kinase Inhibition Is Synthetic Lethal with Loss of the RB1 Tumor Suppressor Gene. Cancer Discov 2019;9:248-63.
57. Vijayaraghavan S, Karakas C, Doostan I, et al. CDK4/6 and autophagy inhibitors synergistically induce senescence in Rb positive cytoplasmic cyclin E negative cancers. Nat Commun 2017;8:15916.
58. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nat Rev Cancer 2017;17:528-42.
59. Shin DW. Dual Roles of Autophagy and Their Potential Drugs for Improving Cancer Therapeutics. Biomol Ther (Seoul) 2020;28:503-11.
60. Narita M, Nũnez S, Heard E, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003;113:703-16.
61. Anders L, Ke N, Hydbring P, et al. A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. Cancer Cell 2011;20:620-34.
62. Kersh AE, Ng S, Chang YM, et al. Targeted Therapies: Immunologic Effects and Potential Applications Outside of Cancer. J Clin Pharmacol 2018;58:7-24.
63. Asghar US, Kanani R, Roylance R, et al. Systematic Review of Molecular Biomarkers Predictive of Resistance to CDK4/6 Inhibition in Metastatic Breast Cancer. JCO Precis Oncol 2022;6:e2100002.
64. Goetz MP, Toi M, Huober J, et al. LBA15 MONARCH 3: Interim overall survival (OS) results of abemaciclib plus a nonsteroidal aromatase inhibitor (NSAI) in patients (pts) with HR+, HER2-advanced breast cancer (ABC). Ann Oncol 2022;33:abstr S1384.
65. Bardia A, Hurvitz SA, Tolaney SM, et al. Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. N Engl J Med 2021;384:1529-41.
66. André F, Nadal J, Denys H, et al. LBA18 Final overall survival (OS) for abemaciclib plus trastuzumab+/- fulvestrant versus trastuzumab plus chemotherapy in patients with HR+, HER2+ advanced breast cancer (monarcHER): A randomized, open-label, phase II trial. Ann Oncol 2022;33:abstr S1386-7.
67. Bidard FC, Hardy-Bessard AC, Dalenc F, et al. Switch to fulvestrant and palbociclib versus no switch in advanced breast cancer with rising ESR1 mutation during aromatase inhibitor and palbociclib therapy (PADA-1): a randomised, open-label, multicentre, phase 3 trial. Lancet Oncol 2022;23:1367-77.

68. Lu YS, Mohd Mahidin EIB, Azim H, et al. Primary results from the randomized Phase II RIGHT Choice trial of premenopausal patients with aggressive HR+/HER2– advanced breast cancer treated with ribociclib + endocrine therapy vs physician's choice combination chemotherapy. San Antonio Breast Cancer Symposium 2022.
69. Asghar US, Barr AR, Cutts R, et al. Single-Cell Dynamics Determines Response to CDK4/6 Inhibition in Triple-Negative Breast Cancer. Clin Cancer Res 2017;23:5561-72.
70. O'Leary B, Cutts RJ, Huang X, et al. Circulating Tumor DNA Markers for Early Progression on Fulvestrant With or Without Palbociclib in ER+ Advanced Breast Cancer. J Natl Cancer Inst 2021;113:309-17.
71. Kalinsky K, Accordino MK, Chiuzan C, et al. A randomized, phase II trial of fulvestrant or exemestane with or without ribociclib after progression on anti-estrogen therapy plus cyclin-dependent kinase 4/6 inhibition (CDK 4/6i) in patients (pts) with unresectable or hormone receptor–positive (HR+), HER2-negative metastatic breast cancer (MBC): MAINTAIN trial. J Clin Oncol 2022;40:abstr LBA1004.
72. Bidard FC, Kaklamani VG, Neven P, et al. Elacestrant (oral selective estrogen receptor degrader) Versus Standard Endocrine Therapy for Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Results From the Randomized Phase III EMERALD Trial. J Clin Oncol 2022;40:3246-56.
73. Lindeman GJ, Fernando TM, Bowen R, et al. VERONICA: Randomized Phase II Study of Fulvestrant and Venetoclax in ER-Positive Metastatic Breast Cancer Post-CDK4/6 Inhibitors - Efficacy, Safety, and Biomarker Results. Clin Cancer Res 2022;28:3256-67.
74. Rugo HS, Chia S, Castan JC, et al. Long-Term and Very-Long-Term Disease Control in Patients From BYLieve Study Cohort A With PIK3CA-Mutant, Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer. San Antonio Breast Cancer Symposium 2022.
75. Xu T, Sun D, Chen Y, et al. Targeting mTOR for fighting diseases: A revisited review of mTOR inhibitors. Eur J Med Chem 2020;199:112391.
76. Rugo HS, Bardia A, Marmé F, et al. Primary results from TROPiCS-02: A randomized phase 3 study of sacituzumab govitecan (SG) versus treatment of physician's choice (TPC) in patients (Pts) with hormone receptor–positive/HER2-negative (HR+/HER2-) advanced breast cancer. J Clin Oncol 2022;40:abstr LBA1001.

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