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Review

Advances in the mechanism of CDK4/6 inhibitor resistance in HR+/HER2– breast cancer

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Sijia Wu, Junnan Xu, Yiwen Ma, Guilian Liang, Jiaxing Wang and Tao Sun

***Abstract*:** Among women, breast cancer is the most prevalent form of a malignant tumour. Among the subtypes of breast cancer, hormone receptor (HR) positive and human epidermal growth factor receptor (HER2) negative kinds make up the biggest proportion. The advent of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors, which are dependent on cell cycle proteins, has greatly enhanced the prognosis of patients with advanced HR+/HER2– breast cancer. This is a specific treatment that stops the growth of cancer cells by preventing them from dividing. Nevertheless, the drug resistance of the disease unavoidably impacts the effectiveness of treatment and the prognosis of patients. This report provides a thorough analysis of the current research advancements about the resistance mechanism of CDK4/6 inhibitors in HR+/HER2– breast cancer. It presents an in-depth discussion from numerous viewpoints, such as aberrant cell cycle regulation and changes in signalling pathways. In response to the drug resistance problem, subsequent treatment strategies are also being explored, including switching to other CDK4/6 inhibitor drugs, a combination of novel endocrine therapeutic agents, an optimal combination of targeted therapies and switching to chemotherapy. An in-depth study of the resistance mechanism can assist in identifying creative tactics that can overcome or postpone drug resistance, alleviate the problem of restricted treatment strategies following drug resistance and enhance the prognosis of patients.

Keywords: breast cancer, CDK4/6 inhibitors, drug resistance, endocrine therapy, hormone receptor-positive, targeted therapy

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Introduction

Breast cancer is a significant worldwide health problem, as it ranks as the second most common form of cancer and is a prominent source of cancer-related fatalities among women.[1] Clinically, based on oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2) and ki-67 status are separated into: Luminal A and Luminal B, HER2-positive (HER2+) and triple-negative breast cancer. Hormone receptor-positive (HR+)/HER2– breast tumours are referred to as Luminal A and Luminal B (HER2-negative) breast cancers.[2] The most prevalent subtype among all subtypes is HR+/HER2, accounting for 74% of cases.[3] Clinically, early-stage breast cancer is treated with surgery, radiotherapy or chemotherapy, depending on the patient's health, tolerance and disease severity.[4] Studies have demonstrated that multimodal therapy has a substantial positive impact on the outlook for individuals diagnosed with early-stage breast cancer, resulting in a 70%–80% increase in their chances of survival. Nevertheless, there is a dearth of efficacious chemotherapy protocols for advanced breast cancer.[5] HR+/HER2– breast cancer is strongly associated with oestrogen, which promotes the growth and spread of tumour cells.[6] Endocrine therapy

Correspondence to:
Tao Sun
Breast Medicine Section One, Liaoning Cancer Hospital, Shenyang, Liaoning 110000, China
jianong@126.com

Sijia Wu
Junnan Xu
Yiwen Ma
Guilian Liang
Jiaxing Wang
Breast Medicine Section One, Liaoning Cancer Hospital, Shenyang, Liaoning, China

inhibits tumour cell proliferation by reducing oestrogen production, modulating ER signalling and/or antagonizing and degrading ER itself.[7] With the continuous advancement of endocrine therapy, the treatment outcome of HR+/HER2− breast cancer has improved significantly. However, a significant percentage of patients will still experience treatment resistance and disease recurrence, which greatly affects survival and quality of life.

The introduction of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors has revolutionized the treatment approach for HR+/HER2− breast cancer.[8] CDK4/6 inhibitors are a specific category of therapeutic medicines that target a specific enzyme known as CDK4/6, by inhibiting the intracellular CDK4 and CDK6 proteins, impeding retinoblastoma protein (RB) phosphorylation and dissociation of the RB/E2F complex by inhibiting intracellular CDK4 and CDK6 proteins, which causes tumour cells to stagnate in the G1 phase, thereby inhibiting tumour cell proliferation (refer to Figure 1). In HR+/HER2− breast cancer, activation of the ER pathway leads to activation of cyclinD and CDK4/6, contributing to tumour expression of malignancy. CDK4/6 inhibitors are efficacious in decreasing the probability of tumour formation.[9] However, CDK4/6 inhibitors still face important challenges in clinical application due to the presence of drug resistance. This review specifically examines the mechanisms by which CDK4/6 inhibitors are resisted in HR+/HER2− breast cancer, as well as prospective therapeutic approaches to counteract this resistance.

Current status of CDK4/6 inhibitor therapy in HR+/HER2− breast cancer

Several recent clinical trials, such as PALOMA,[10] DAWNA[11] and MONALEESA,[12] have assessed the effectiveness and safety of CDK4/6 inhibitors paired with ET in HR+/HER2− breast cancer (refer to Table 1). CDK4/6 inhibitors in combination with ET have emerged as the most effective standard treatment for HR+/HER2− breast cancer. The combination therapy has demonstrated significant efficacy in China, where the use of CDK4/6 inhibitor therapy is advised for HR+/HER2− advanced breast cancer according to the CSCO (GUIDELINES OF CHINESE SOCIETY OF CLINICAL ONCOLOGY, CSCO) Breast Cancer Diagnostic and Treatment Guidelines.[13] The four CDK4/6 inhibitors, namely palbociclib, abemaciclib, ribociclib, dalpiciclib, have been authorized for marketing in China. These inhibitors have demonstrated substantial enhancements in progression-free survival (PFS) in clinical therapy studies for advanced breast cancer, both in the first-line and second-line settings. CDK4/6 inhibitors are becoming more commonly utilized in the management of individuals with advanced breast cancer as research advances.

Despite the significant advancements made in the prognosis of patients with HR+ breast cancer with the use of targeted inhibitors of CDK4/6, these medicines are still susceptible to primary or secondary resistance. Primary resistance occurs in approximately 10% of patients, and it takes about 24–28 months for secondary resistance to develop, even after undergoing primary chemotherapy; in second-line therapy, the time for secondary resistance to occur is even shorter.[14] Given the limited treatment options available after resistance occurs, it is crucial to gain a precise understanding of the resistance mechanism of CDK4/6 inhibitors and to investigate appropriate therapy techniques. This has become a significant area of research focus.

Mechanisms of resistance to CDK4/6 inhibitors

Cell cycle-specific resistance mechanisms (refer to Figure 2)

Altered activity of the CDK4/6-cyclinD complex

CDK4/6 amplification. CDK4 or CDK6 amplification is believed to be a significant factor in developing resistance to CDK4/6 inhibitors. CDK4/6 plays a key role in the G1 to S phase transition of the cell cycle, and its amplification activates the cyclinD-CDK4/6-RB pathway, which drives cell cycle progression and thus reduces the blocking effect of CDK4/6 inhibitors on the cell cycle.[15] Multiple studies have demonstrated a substantial increase in the phosphorylation of RB proteins in cells that overexpress CDK4/6, leading to dissociation of the RB-E2F complex, which drives cell cycle progression. This process reduces the sensitivity to CDK4/6 inhibitors and facilitates the development of medication resistance.[16,17] According to Cornell et al., drug-resistant breast cancer cells can inhibit the Transforming Growth Factor-β (TGF-β)/Smad4 protein (SMAD4) pathway via miR-432-5p, thereby increasing CDK6 expression. In this mechanism, miR-432-5p mediates the transmission of the drug-resistant

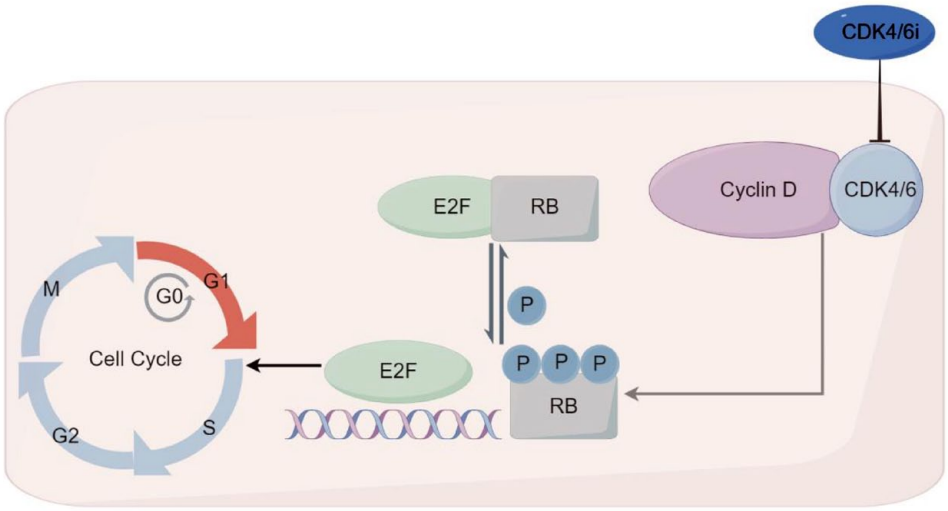

Figure 1. Schematic diagram of the mechanism of action of CDK4/6 inhibitors in HR+/HER2– breast cancer cell cycle. CDK4/6 inhibitors impede the activity of the CDK4/6 protein in cancerous cells, hindering the phosphorylation of RB and the dissociation of the RB/E2F complex. Consequently, these inhibitors restrict the proliferation of tumour cells during the G1 phase.
CDK4/6, cyclin-dependent kinase 4 and 6; HER, human epidermal growth factor receptor 2; HR, hormone receptor; RB, retinoblastoma protein.

phenotype between neighbouring cells through exosomal expression.[18] In addition, CDK6 can regulate Vascular Endothelial Growth Factor-A (VEGF-A) expression through c-Jun and promote neoangiogenesis, thereby promoting tumour progression and drug resistance.[19]

P16 amplification. p16 is a protein encoded by the *CDKN2A* gene, one of the members of the INK4 family of CDK inhibitors, and is an important tumour suppressor. p16 inhibits the formation of the cyclinD-CDK4/6 complex and CDK4/6-mediated phosphorylation of RB by binding to CDK4/6, maintaining RB in a low phosphorylated state and enhancing its interaction with E2F which, in turn, blocks the G1 phase and weakens or eliminates the effects of CDK4/6 inhibitors.[20] In addition, studies have shown that the expression of p16 protein is significantly higher in drug-resistant breast cancer than in sensitive breast cancer.[21] All of these findings imply that the amplification of p16 is closely connected with medication resistance to CDK4/6 inhibitors.

Overexpression of the cyclinE-CDK2 complex. The cyclinE-CDK2 complex is also capable of driving cells from the G1 phase into the S phase by a mechanism similar to that of cyclinD-CDK4/6. Thus, aberrant activation of the cyclinE-CDK2 complex is capable of fully phosphorylating RB and releasing E2F, thereby bypassing CDK4/6 inhibition and allowing cells to enter S phase.[22] Several studies have shown that overexpression of cyclinE1 is usually present in CDK4/6 inhibitor-resistant cells.[23,24] In addition, when CDK2 was inhibited, the overexpression effect of cyclinE disappeared and the combination of CDK2/4/6 inhibitors and endocrine therapy could effectively inhibit cell growth.[24] Studies have shown that the non-selective CDK2 inhibitor dinaciclib has better efficacy in combination with palbociclib and letrozole,[24] which provides direction for the development of subsequent combination therapy strategies.

p27, acting as a tumour suppressor, has a role in several biological processes such as cell proliferation, differentiation, migration and death.[25] p21 and p27 can attach to and hinder the cyclin E-CDK2 and cyclin A-CDK2 complexes, resulting in the cessation of the cell cycle in the G1 phase.[26] The anaphase-promoting complex, cyclosome (APC/C) ubiquitin ligase can be activated by Fizzy and cell division cycle 20-related 1 (FZR1) to create the APC/C-FZR1 complex,[27] which interacts with RB during the G1 phase interacts with RB, leading to cell cycle arrest; it also degrades S-phase kinase-associated proteins, which, in turn, further inhibits p27, leading to the reduction of CDK2, CDK4 and CDK6.[27] Ultimately, FZR1

Table 1. Selected studies testing CDK4/6 inhibitors in patients with metastatic hormone receptor-positive HER2– breast cancer.

Trial name	Setting	Population	Treatment arms	Sample size	Primary outcome (Exp vs Ctrl Arm) HR (95% CI)
PALOMA-1	Advanced or metastatic	AI-sensitive postmenopausal women with HR-positive/ HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC	Palbociclib + letrozole vs letrozole	165	PFS 20.2 vs 10.2 months (HR 0.488; 95% CI: 0.319–0.748)
PALOMA-2	Advanced or metastatic	AI-sensitive postmenopausal women with HR-positive/ HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC	Palbociclib + letrozole vs letrozole	666	PFS 27.6 vs 14.5 months (HR 0.563; 95% CI: 0.461–0.687)
PALOMA-3	Advanced or metastatic	AI-resistant pre/ postmenopausal women with HR-positive/HER2-negative advanced or metastatic breast cancer that progressed after ET	Palbociclib + fulvestrant vs fulvestrant + Placebo	521	PFS 9.5 vs 4.6 months (HR 0.46; 95% CI: 0.36–0.59)
PEARL	Advanced or metastatic	AI-resistant postmenopausal women with HR-positive, HER2-negative metastatic BC	Palbociclib + ET vs capecitabine	601	PFS 7.5 vs 10 months (HR 1.09; 95% CI: 0.83–1.44)
MONARCH-2	Advanced or metastatic	AI-resistant pre/ postmenopausal women with HR-positive/HER2-negative advanced BC that progressed after ET; no previous CT for ABC	Abemaciclib + fulvestrant vs placebo + fulvestrant	669	PFS 16.4 vs 9.3 months (HR 0.553; 95% CI: 0.449–0.681)
MONARCH-3	Advanced or metastatic	AI-sensitive postmenopausal women with HR-positive/ HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC	Abemaciclib + NSAI vs placebo + NSAI	493	PFS 28.1 vs 14.7 months (HR 0.540; CI: 0.418–0.698)
MONALEESA-2	Advanced or metastatic	AI-sensitive postmenopausal women with HR-positive/ HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC	Ribociclib + letrozole vs letrozole + placebo	668	PFS 25.3 vs 16 months (HR 0.568; 95% CI: 0.457–0.704)
MONALEESA-3	Advanced or metastatic	AI-sensitive/-resistant postmenopausal women with HR-positive/HER2-negative advanced or metastatic BC; 0–1 line of ET for ABC	Ribociclib + fulvestrant vs fulvestrant + placebo	726	PFS 20.5 vs 12.8 months (HR 0.593; 95% CI: 0.480–0.732)
MONALEESA-7	Advanced or metastatic	AI-sensitive peri/ premenopausal women with HR-positive/HER2-negative advanced or metastatic BC; no previous ET and up to 1 line of CT for ABC	Ribociclib + TAM/NSAI vs TAM or NSAI + placebo	672	PFS 23.8 vs 13.3 months (HR 0.553: 95% CI: 0.441–0.694)

(Continued)

Table 1. (Continued)

Trial name	Setting	Population	Treatment arms	Sample size	Primary outcome (Exp vs Ctrl Arm) HR (95% CI)
MAINTAIN	Advanced or metastatic	Pre/postmenopausal women or men with HR-positive/ HER2-negative advanced or metastatic BC who have progressed on an AI plus a CDK4/6 inhibitor (either palbociclib or ribociclib)	Ribociclib + fulvestrant vs fulvestrant + placebo	109	PFS 5.29 vs 2.76 months (HR 0.57: 95% CI: 0.39–0.85)
DAWNA-1	Advanced or metastatic	Endocrine therapy-sensitive pre/postmenopausal women with HR-positive/ HER2-negative advanced or metastatic BC	Dalpiciclib + fulvestrant vs fulvestrant + placebo	361	PFS 15.7 vs 7.2 months (HR 0.42: 95% CI: 0.31–0.58)
DAWNA-2	Advanced or metastatic	Endocrine therapy-sensitive pre/postmenopausal women with HR-positive/ HER2-negative advanced or metastatic BC	Dalpiciclib + letrozole vs letrozole + placebo	456	PFS 30.6 vs 18.2 months (HR 0.51: 95% CI: 0.38–0.69)

ABC, Advance Breast Cancer; AI, Aromatase Inhibitors; BC, Breast Cancer; CDK4/6, cyclin-dependent kinase 4 and 6; CI, confidence interval; ET, Endocrine Therapy; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; NSAI, Non-Steroidal Aromatase Anhibition; PFS, progression-free survival; TAM, Tamoxifen.

deficiency promotes the transition from G1 to S phase. Conversely, when FZR1 is phosphorylated, it loses its capacity to activate APC/C. FZR1 functions as a substrate similar to cyclin D-CDK4/6.[27] Consequently, the removal of FZR1 could result in resistance to CDK4/6 inhibitors, although the precise mechanism behind this resistance still requires additional investigation.

Inhibition of the p53-p21-RB pathway. p53, functioning as a transcription factor, has a significant impact on cell cycle cessation, senescence, DNA mending and programmed cell death.[28] p21 protein is an important target of p53, which binds to and activates the region in the p21/CDKN1A promoter.[29] p21 hinders the activity of different G1-phase cyclin-CDK complexes that are responsible for phosphorylating RB, hence impeding the advancement of the cell cycle. RB which is not phosphorylated interacts with E2F to create a complex that inhibits transcription by attaching to E2F-binding sites in the promoters of target genes. The p53-p21-RB transduction mechanism, comprised of these components, facilitates cell cycle arrest and apoptosis.[30]

Mouse double minute 2 homolog (MDM2) oncogene is a protein that negatively regulates p53 activity.[31] Under normal physiological conditions, MDM2 can inhibit the function of p53 by binding directly to the transcriptional activation region of p53 or ubiquitinating it. Simultaneously, p53 can control MDM2 expression by attaching itself to its promoter, resulting in the creation of a negative feedback loop.[32] Therefore, the overexpression of MDM2 can hinder DNA repair, facilitate improper cell cycle advancement and impede apoptosis in cells that have suffered significant damage.[33] CDK4/6 inhibitor-resistant cells are unable to efficiently induce senescence due to the disruption of the p53 signalling pathway caused by the upregulation of MDM2, which leads to drug resistance.[31] The first MDM2 inhibitor, NVP-CGMM097, has entered phase I clinical trials,[34] and in the future, MDM2 may become a new target for treating CDK4/6 inhibitor resistance.

Histone deacetylases (HDACs) act as epigenetic modifiers that inhibit p21.[35] Zhou et al. demonstrated that HDAC5 interacts with RB and histones to remove acetyl groups from them, resulting in the suppression of gene expression linked with cell cycle progression and promoting carcinogenesis, ultimately leading to inhibition of cell growth. Furthermore, CDK4/6 inhibitors

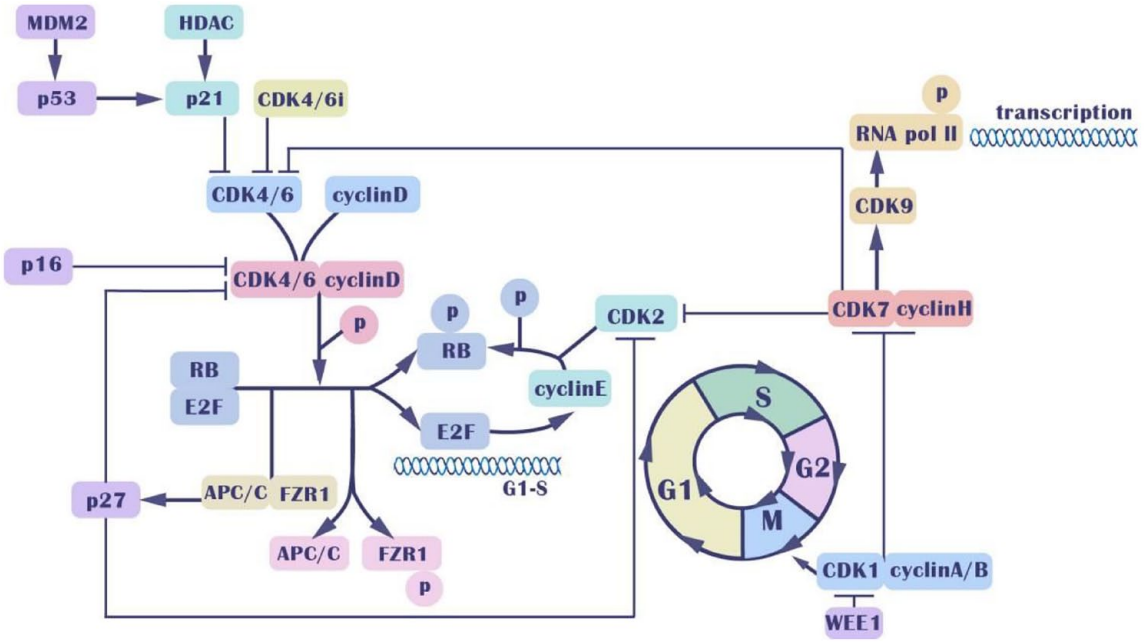

Figure 2. Schematic diagram of cell cycle-specific drug resistance mechanisms.
Multiple regulatory factors involved in the cell cycle are associated with CDK4/6 inhibitor resistance.
APC/C, anaphase-promoting complex, cyclosome; CDK, cyclin-dependent kinase; FZR1, Fizzy and cell division cycle 20-related 1; HDAC, histone deacetylases; MDM2, mouse double minute 2 homolog; RB, retinoblastoma protein.

were capable of enhancing this binding impact. Nevertheless, solid tumours often lack HDAC5, which results in resistance to CDK4/6 inhibitors. This resistance occurs because the absence of HDAC5 disrupts the ability of palbociclib to induce histone deacetylation and suppress the production of oncogenes.[36] HDAC can also inhibit the efficacy of CDK4/6 inhibitors by increasing chromatin condensation to repress p21.[37] The HDAC inhibitor, tucidinostat in combination with exemestane showed promotion of anti-breast cancer activity.[38] In a clinical trial conducted by Zhou et al.[39] in 2022, it was found that the median PFS after administering consecutive tucidinostat in combination with endocrine therapy following treatment with CDK4/6 inhibitors was 4.5 months. The study suggests that HDAC is expected to be a new therapeutic target for the treatment of CDK4/6 inhibitor resistance.

Overexpression of CDK7 and activation of CDK9. CDK7 is seen as a promising target in cancer therapy because of its ability to regulate both the cell cycle and transcription.[40] It binds to cyclin H and the auxiliary protein MAT1,[41] functions as a CDK-activating kinase, participates in G1 and G2 phases by maintaining CDK1/2/4/6 activity and promotes cell-cycle progression leading to drug resistance.[42] In a pilot review conducted by Coombes et al., it was found that the CDK7 inhibitor samuraciclib exhibited a clinical response rate of 36% (9 out of 25 patients) and a median PFS of 3.7 months in patients who had received a combination of a CDK4/6 inhibitor and fulvestrant. These data imply that samuraciclib may be beneficial in decreasing disease progression caused by CDK4/6 inhibitor resistance.[43]

On the other hand, CDK7 is activated upon binding to cyclin H and MAT1, which further promotes phosphorylation of the Ser5 and Ser7 sites of RNA polymerase II, thereby contributing to the dissociation of the preinitiation complex by RNA polymerase II and driving transcription initiation.[44] CDK7 promotes RNA polymerase II phosphorylation and transcription through activation of the downstream transcriptional regulator CDK9 elongation.[45] 2023 Soosainathan's study showed that pathway analysis confirmed RNA polymerase II-mediated transcriptional down-regulation after treatment of ER+ mammary cancer mice with the CDK9 inhibitor AZD4573. In tumours treated with palbociclib in combination with fulvestrant, the downregulated pathway was revealed to be enriched for cell cycle

control pathways in the absence of AZD4573. The emergence of palbociclib resistance may be linked to a heightened reliance on CDK9 function. Consequently, the administration of AZD4573 in conjunction with palbociclib and fulvestrant can result in the regression of tumours.[46] At present, there are ongoing research efforts to develop small molecule inhibitors that can effectively inhibit CDK9. These inhibitors can be classified into two generations: first-generation inhibitors such as flavopiridol, dinaciclib and seliciclib, and second-generation inhibitors such as atuveciclib, AZD4573, i-CDK9 and NVP-2. CDK9's function in promoting resistance to CDK4/6 inhibitors. Additional research is required to investigate the molecular pathways.[45]

CDK1 inhibition. CDK1 phosphorylates downstream signalling factors at different stages of the cell cycle, hence promoting the progression of the cell cycle.[47] WEE1 is a pivotal tyrosine kinase that plays a crucial role in regulating the G2/M and S phases of the cell cycle.[48] WEE1 synergistically controls the entry of DNA-damaged cells into mitosis with CDK1.[49] While the specific process by which WEE1 causes resistance to CDK4/6 inhibitors is not yet understood, Fallah's experimental research demonstrated that using AZD1775, a small molecule inhibitor of WEE1, led to a notable decrease in the quantity of drug-resistant cells. This reduction promotes cell cycle arrest and apoptosis specifically in the G2/M phase.[50] Therefore, WEE1 combined with CDK4/6 inhibitors targeting G2/M phase cells may be one of the therapeutic options to overcome drug resistance.

RB deletion and E2F overexpression. RB serves as an important downstream target in the chain of action of CDK4/6 inhibitors. A variety of HR+/HER2− breast cancer cells show reduced RB expression when induced to develop CDK4/6 inhibitor resistance.[51] Despite reduced RB expression, the cell cycle can continue through the activation of other cell cycle mechanisms, such as E2F and the cyclin-CDK2 axis.[52] A comprehensive CRISPR screen throughout the entire genome showed that suppressing the activity of protein arginine methyltransferase 5 (PRMT5) in ER-positive breast tumours without the RB halted the transition from the G1 phase to the S phase. Furthermore, this suppression of PRMT5 function was observed to occur autonomously in cells lacking RB. PRMT5 inhibitors synergized with fulvestrant that inhibits the growth of RB-deficient HR+/HER2− breast cancers are expected to be a new therapeutic strategy,[53] which provides a new option for the post-resistance therapeutic strategy of CDK4/6 inhibitors.

Due to the multitude of E2F targets, it can participate in tumourigenesis and progression through a variety of mechanisms. Irrespective of the condition of RB, an elevation in E2F can override the suppression of CDK4/6 and directly stimulate DNA replication and mitosis, resulting in the development of resistance to CDK4/6 inhibitors.[54]

Alterations in non-specific mechanisms of the cell cycle (refer to Figure 3)

Activation of fibroblast growth factor receptor 1. The fibroblast growth factor receptor (FGFR) family, which consists of highly conserved transmembrane receptors (FGFR1–4), has a significant oncogenic impact on breast cancer. It controls crucial biological processes such as cell proliferation, migration, invasion and angiogenesis.[55] Research has demonstrated that FGFR1 and FGFR2 are not only linked to endocrine resistance but also to resistance against CDK4/6 inhibitors.[56] Research has shown that FGFR1 amplification triggers the activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway and mitogen-activated protein kinase (MAPK) in breast cancer cells that are resistant to treatment. The kinase (MAPK) pathways are activated, leading to the restoration of the ability of tumour cells to proliferate.[57] The ctDNA analysis results from the MONALEESA-2 study indicate that patients with FGFR1 amplification had a significantly shorter PFS compared to individuals with wild-type FGFR1.[58] It was reported that the combination of tyrosine kinase inhibitor lucitanib (targeting FGFR) to palbociclib/fulvestrant elicited full remission in FGFR1 amplified ER+ patient-derived xenografts.[58] The above suggests that combined blockade of the FGFR pathway by CDK4/6 inhibitors could be one of the strategies to overcome drug resistance.

Activation of the MAPK pathway. Breast cancer and the MAPK pathway are closely related.[59] KRAS mutations account for 86% of all RAS pathway aberrations and are present in approximately 20% of the population.[60] Research has demonstrated that the majority of patients with KRAS-mutant malignancies exhibit elevated levels of cyclinD1 expression. Additionally, the

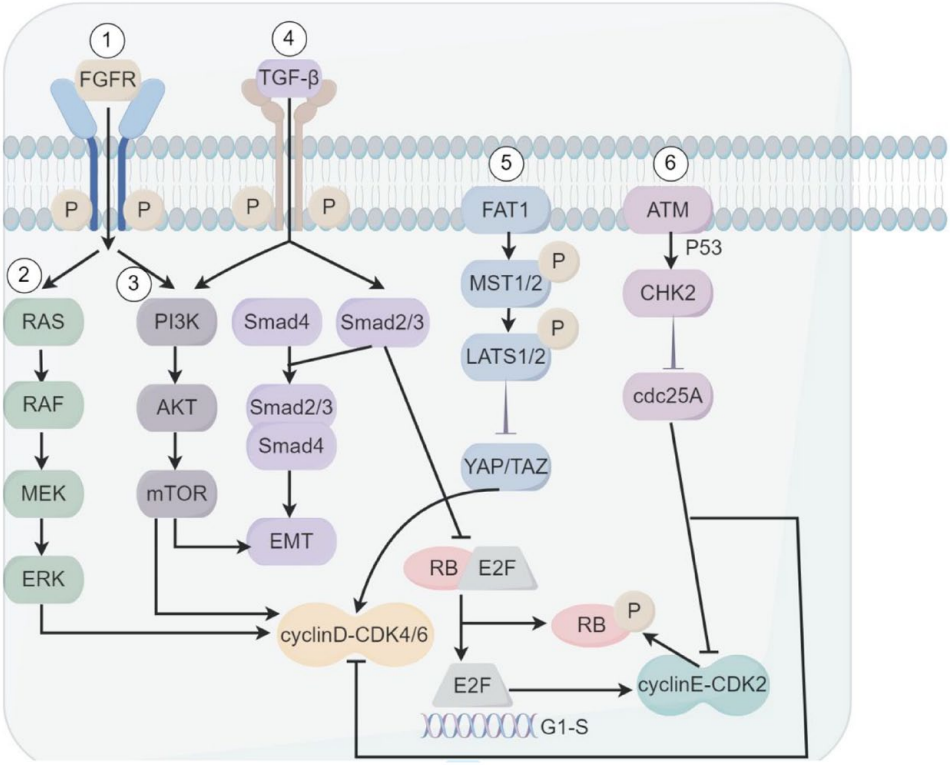

Figure 3. Schematic diagram of nonspecific resistance mechanisms of CDK4/6 inhibitors. (1) Activation of FGFR1. (2) Activation of the MAPK pathway. (3) Activation of the PI3K/AKT/mTOR pathway. (4) Activation of the EMT pathway. (5) Hippo pathway inhibition. (6) Activation of the ATM-CHK2 pathway.
CDK4/6, cyclin-dependent kinase 4 and 6; EMT, epithelial–mesenchymal transition; FGFR, fibroblast growth factor receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase.

activation of RAS family oncogenes (*KRAS*, *HRAS*, *NRAS*) has been observed in tumour biopsy samples taken from patients who are resistant to CDK4/6 inhibitors.[61] This explains that KRAS produces aberrant growth signals in the presence of CDK4/6 inhibitors, leading to cellular resistance.[62]

Activation of the PI3K/AKT/mammalian target of rapamycin pathway. The PI3K/AKT/mammalian target of rapamycin (mTOR) pathway targets plays a crucial role in various essential biological functions, including cell growth, proliferation, metabolism and survival.[63] Vilgelm et al. have reported that the PI3K/AKT pathway is activated in cells that are resistant to CDK4/6 inhibitors. The PI3K/AKT pathway inactivates p21 and p27, while CDK4/6 inhibitors amplify cyclinD in tumour cells, which binds to the downstream regulators of the pathway, p21 and p27. When these two CDK inhibitors are inhibited, they will not bind to CDK2, allowing the cells to proliferate. Furthermore, the restoration of the functional significance of p21 in CDK4/6 inhibitors was achieved by employing a particular siRNA for p21. This restoration resulted in the sensitivity of tumour cells to CDK4/6 inhibitors being restored as well. This confirms that the activation of the PI3K-AKT pathway and resistance to CDK4/6 inhibitors may be interconnected.[64] Michaloglou et al.[65] found that mTORC1/2 inhibitors reduced E2F-mediated transcription, suggesting that mTOR-amplified cells can resist the impact of CDK4/6 inhibitors through E2F-mediated transcription, leading to drug resistance.

Activation of the EMT pathway. The EMT pathway refers to the change from endothelium to mesenchymal cells and is one of the causes of cancer therapeutic resistance.[66] CDK4/6 inhibitors stimulate epithelial–mesenchymal transition (EMT) by activating both the TGF-β-Smad pathway and the PI3K/AKT/mTOR pathway. Phosphorylated TGF-β activates Smad2/3, which forms a complex with Smad4, which leads to EMT by activation of EMT transcription factors leading to EMT.[67] Furthermore, TGF-β triggers EMT through the PI3K/AKT/mTOR pathway.[68]

Smad3 inhibition can release the blockade of E2F via the RB-E2F complex, which, in turn, induces the recovery of the cell cycle block.[69] Furthermore, the inhibition of Smad3 is strongly linked to the stimulation of the cyclinE-CDK2 axis, which ultimately results in the emergence of resistance to CDK4/6 inhibitors. Hence, suppressing EMT or enhancing the activity of Smad3 could potentially be utilized as a strategy to overcome the resistance to CDK4/6 inhibitors.

Hippo pathway inhibition. Atypical cadherin 1 (FAT atypical cadherin 1 (FAT1)) is a tumour suppressor that interacts with the Hippo pathway and is involved in tumour development, proliferation, migration and invasion.[70] The Hippo pathway consists of Recombinant Neurofibromin 2 (NF2), protein kinase MST1/2 (MST1/2), large tumour suppressor kinase 1/2 (LATS1/2), Yes-associated protein 1 (YAP1), TAZ protein (Tafazzin, TAZ) and TEA domain transcription factor (TEA domain transcription factor). The stimulation of the Hippo pathway triggers the activation of MST1/2, which, in turn, triggers the phosphorylation of LATS1/2 and subsequently suppresses the activity of YAP1 and TAZ. YAP/TAZ are transcriptional co-activators that interact with TEAD and control the activity of many proteins involved in cell proliferation, programmed cell death and the activation of stem cells. They also influence the expression of genes related to apoptosis and the renewal of stem cells.[71] Mutational deletion of FAT1 function, although rare, has been associated with CDK4/6i resistance.[72] FAT1 deletion reduces Hippo signalling and stimulates the nucleus localization of YAP1/TAZ, which enhances CDK6 expression. Simultaneously, the inactivation of NF2 results in an upregulation of CDK6 expression. These two processes work together to decrease the sensitivity of cells to CDK4/6 inhibitors, resulting in the development of drug resistance. The experimental results of Li et al. showed that parental cells were completely inhibited when exposed to 50 nM abecyclic, whereas cells knocked down or knocked down FAT1 were not inhibited. These findings demonstrate an association between FAT1 deletion and an attenuated response to abecyclidine.[73] Therefore, FAT1 could potentially function as a biomarker for the development of resistance to CDK4/6 inhibitors.

Activation of the ATM-CHK2 pathway. The ATM protein plays a crucial function in controlling the G1/S checkpoint. Upon activation, ATM activates CHK2 through activation of p53, which inhibits the activity of Cdc25A and Cdc25C, impeding the progression of the cell cycle.[74] Cdc25A exhibits dual-specificity phosphatase activity, which dephosphorylates CDK2, CDK4 and CDK6 and promotes their activation to drive cell cycle progression.[75] Phosphorylation of CHK2 promotes the degradation of Cdc25A through the proteasome, which inhibits the process of dephosphorylation and activation of CDK2. Therefore, the ATM-CHK2-Cdc25A and cyclinD-CDK4/6-RB pathways may have a mutual influence on the other, resulting in the development of resistance to CDK4/6 inhibitors.[76]

Other mechanisms

Immunological mechanisms

CDK4/6 inhibitors exert an influence on the expression of several cytokines, including PD-L1, Major Histocompatibility Complex-I (MHC-I) and T cells, by altering the tumour microenvironment. Studies have indicated that CDK6 may mediate immunological escape. The nuclear factor of activated T cells (NFAT) is a family of transcription factors that play a key role in triggering gene transcription in the immune response.[77] NFAT stimulates T cells and enhances the process of IL-2 transcription, while CDK6 phosphorylates and hinders the function of NFAT4, leading to a reduction in IL-2 levels. On the other hand, CDK4/6 inhibitors raise IL-2 levels by removing phosphate groups from NFAT4 and boosting its effectiveness, so stimulating the body's immune response against tumours.[78] The cyclin D/CDK4 complex facilitates the breakdown of the PD-L1 protein through the Cullin3-SPOP E3 ubiquitin ligase (refer to Figure 4). Palbociclib hinders the process of SPOP-mediated ubiquitinated degradation of PD-L1 by inhibiting cyclin D/CDK4. This leads to increased levels of PD-L1 in in vivo models and primary human prostate cancer specimens. Administering a CDK4/6 inhibitor alongside anti-PD-1 immunotherapy improves the regression of tumours. This indicates that combining CDK4/6 inhibitor and PD-1/PD-L1 immune checkpoint suppression therapy has the potential to enhance the effectiveness of tumour treatment.[79] DNA methyltransferase (DNA (cytosine-5-)-methyltransferase 1 (DNMT)) is a protein that targets E2F and helps cytotoxic T cell-mediated tumour suppression. Research has demonstrated that CDK4/6 inhibitors hinder the growth of tumours by decreasing

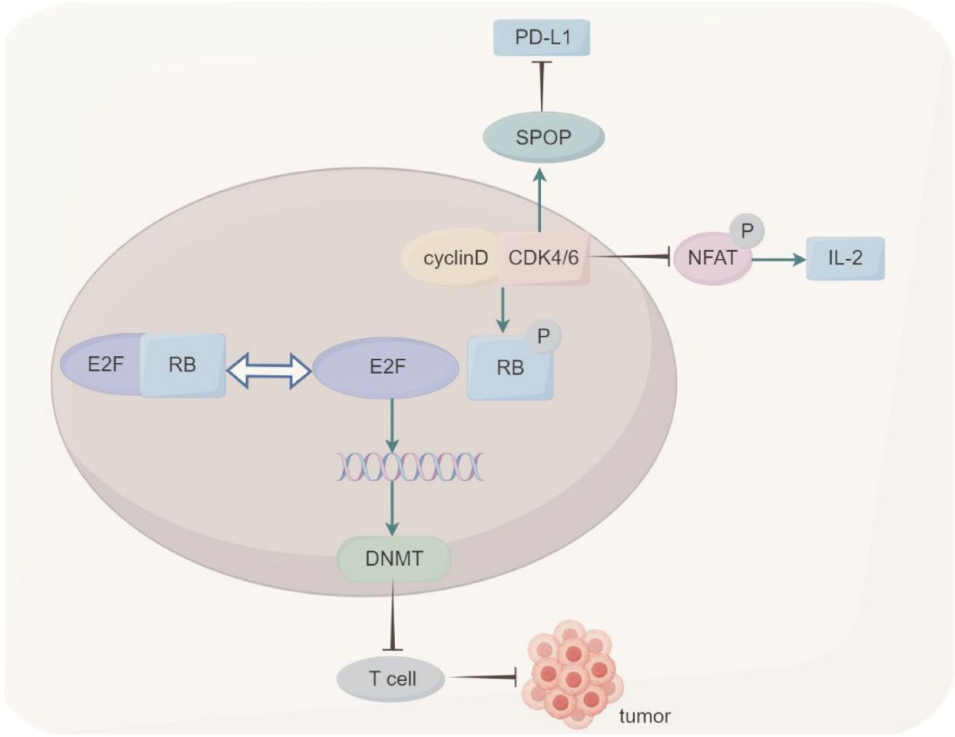

Figure 4. Schematic diagram of tumour immune mechanism.
CDK6 phosphorylates the nuclear factor of NFAT4 and inhibits its activity, resulting in a decrease in IL-2 levels. The cyclin D/CDK4 complex mediates the degradation of PD-L1 protein via Cullin3-SPOP E3 ubiquitin ligase. DNMT, as a target protein of E2F, contributes to cytotoxic T cell-mediated tumour suppression.
CDK4/6, cyclin-dependent kinase 4 and 6; DNMT, DNA (cytosine-5-)-methyltransferase 1; E2F, Early 2 Factor; IL-2, Interleukin-2; NFAT4, nuclear factor of activated T-cells; PD-L1, Programmed cell death-Ligand 1.

the activity of DNMT, an enzyme responsible for DNA methylation.[80]

The PD-L1 inhibitor navumab in combination with abciximab was found to enhance the inflammatory response and promote T-lymphocyte proliferation,[81] according to a non-randomized, multi-cohort, phase II clinical trial study from 2023. CDK4/6 inhibitors can also lead to drug resistance through immune mechanisms. Although initially cancer cells can be effectively monitored and recognized by the immune system, due to the immune escape mechanism of cancer, this can eventually lead to the tumour entering an immune escape state, which uses the immune system to promote faster metastasis.[82] Studies have shown that interferon (IFN) signalling is a key pathway associated with CDK4/6 inhibitor resistance. IFN signalling is overactive in piperacilli resistance models, but the data results do not support a direct correlation between the activation of IFN signalling and RB deletion. The IFN-associated piperacilli resistance signature gene set was associated with M1-polarized macrophages, regulatory T-cell infiltration and immune checkpoint expression. It was hypothesized that IFN signalling may contribute to the immunosuppressive microenvironment by enhancing the expression of immune checkpoints and regulatory T-cell infiltrates, as well as by altering the classical function of M1 macrophages.[83] IFN signalling was shown to be associated with resistance to abemaciclib in combination with an aromatase inhibitor in the whole genomics of clinical samples from breast cancer patients in the NeoMONARCH trial.[84] The data indicate that there is a possibility of developing resistance to CDK4/6 inhibitors through immune responses; however, the specific mechanism requires additional investigation.

Activation of autophagy. Autophagy is a metabolic process that breaks down and disposes of organelles and cellular components by delivering them to lysosomes. This not only eliminates defective or damaged organelles and components but also recycles substrates needed to maintain homeostasis in the body in times of nutrient

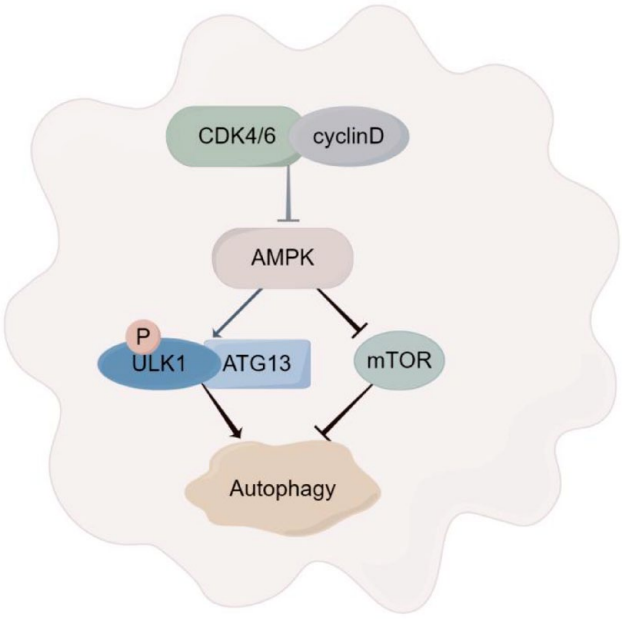

Figure 5. Schematic diagram of autophagy mechanism.
CDK4/6 binding to cyclinD results in AMPK inactivation. Activation of AMPK can induce autophagy through inhibition of mTOR kinase complex and direct phosphorylation of ULK1.
AMPK, adenosine 5′-monophosphate-activated protein kinase; CDK4/6, cyclin-dependent kinase 4 and 6; mTOR, mammalian target of rapamycin; ULK1, unc-51-like kinase 1.

deficiency.[85] Autophagy plays a two-fold role in cancer: firstly, it hinders the advancement of tumours by limiting the build-up of impaired proteins and organelles; secondly, it functions as a cellular survival mechanism that fosters the expansion of existing tumours.[86] The interaction between CDK4/6 and cyclinD triggers the activation of adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) deactivation.[87] AMPK is a protein kinase that is highly conserved throughout evolution. It acts as a detector of cellular energy balance during times of metabolic stress, both at the cellular and physiological levels. Its main function is to restore and maintain cellular energy balance.[88] AMPK activation can be mediated by inhibition of the mTOR kinase complex and direct phosphorylation of ULK1 (mammalian homologue of unc-51-like kinase 1, Atg1) both to induce autophagy process.[89] CDK4/6 activation results in AMPK inactivation, disrupting AMPK's regulation on mTOR; the activation of mTOR subsequently inhibits autophagy (refer to Figure 5).[87] Research has demonstrated that the inhibition of autophagy significantly amplifies the effectiveness of piperacilli in treating breast cancer.[37] Lanceta's work demonstrated a notable augmentation in autophagosome formation when palbociclib was introduced to MCF7 cells. Autophagy contributes to the development of resistance in breast cancer cells against CDK4/6 inhibitors by enhancing the survival of tumour cells and inhibiting apoptosis.[90] Vijayaraghavan et al. found that when the autophagy genes *Beclin-1* and *ag-5* were down-regulated, there was a notable enhancement in the responsiveness of ER+ breast cancer cells to palbociclib. Knocking down both induced permanent growth suppression, blocking the G1 phase and speeding up the process of cellular senescence. In addition, experimental evidence demonstrated that the autophagy inhibitor Hydroxychoroquine (HCQ), when paired with palbociclib, resulted in prolonged and consistent suppression of growth. This finding indicates a synergistic interaction of CDK4/6 inhibitors and autophagy inhibition.[37] Autophagy inhibitors were shown to be effective in overcoming resistance to CDK4/6 inhibitors.

Novel therapeutic options for the follow-up of CDK4/6 inhibitor resistance

For HR+/HER2- breast cancer patients, after the progress of CDK4/6 inhibitor treatment, the main replacement was CDK4/6 inhibitor or

Table 2. Selected clinical trials for the treatment of metastatic HR+/HER2– breast cancer after treatment progression with CDK4/6 inhibitors.

Drug	Trial name	Phase	Treatment arms	Sample size	History of CDK4/6i treatment, %	mPFS
Palbociclib	PACE	II	Palbociclib + fulvestrant vs fulvestrant	220	100	4.6 vs 4.8 m HR, 1.11 (0.79–1.55)
Ribociclib	MAINTAIN	II	Ribociclib + ET vs ET	120	100	5.29 vs 2.76 m HR, 0.57 (0.39–0.85)
Elacestrant	EMERALD	III	Elacestrant vs ET	477	100	ESR1m: 3.78 vs 1.87 m HR, 0.55 (0.39–0.77)
Camizestra	SERENA-2	II	Camizestrant vs fulvestrant	240	50	7.2–7.2 vs 3.7 m
Alpelisib	SOLAR-1	III	Alpelisib + fulvestrant vs fulvestrant	572	5.9	11.0 vs 5.7 m HR, 0.65 (0.50–0.85)
Alpelisib	BYLieve	II	Alpelisib + fulvestrant vs letrozole	336	100	8.2 vs 5.6 m
Capivasertib	CAPItello-291	III	Capivasertib + fulvestrant vs fulvestrant	708	69	7.2 vs 3.6 m HR, 0.60 (0.51–0.71)
Everolimus	BOLERO-2	III	Everolimus + exemestane vs exemestane	724	0	10.6 vs 4.1 m
DS-8201	DB-04	III	T-DXd vs TPC	557	70.4	10.1 vs 5.4 m HR, 0.51 (0.40–0.61)
Sacituzumab govitecan	TROPICS-02	III	T-DXd vs TPC	543	100	5.5 vs 4.0 m HR, 0.55 (0.53–0.83)
Olaparib	OlympiAD	III	Olaparib vs chemotherapy	302	0	7.0 vs 4.2 m HR, 0.58 (0.43–0.80)
Talazoparib	EMBRACA	III	Talazoparib vs haemotherapy	431	0	8.6 vs 5.6 m HR, 0.542 (0.413–0.71)
Tucidinostat	ACE	III	Tucidinostat + exemestane vs Placebo + exemestane	365	0	7.4 vs 3.8 m HR, 0.75 (0.58–0.98)

CDK4/6, cyclin-dependent kinase 4 and 6; ESR1m,estrogen receptor α mutation; ET, endocrine therapy; HR, hazard ratio; HER2–, human epidermal growth factor receptor-2; mPFS, progression-free survival.

endocrine drugs, new endocrine therapy, other targeted drugs and chemotherapy. Clinical trial data supporting these options are detailed in Table 2.

Replacement of CDK4/6 inhibitors or endocrine drugs

The MonarchE[91] and next MONRCH[92] studies have substantiated the effectiveness of abemaciclib

when used in conjunction with ET treatment. Now according to a multicentre retrospective study in 2020, it was found that after prior treatment with palbociclib, followed by abemaciclib was well tolerated with a median PFS of 5.3 months and a median overall survival (OS) of 17.2 months. Patients who were administered CDK4/6 inhibitors in a sequential manner experienced a significantly longer median PFS of 8.4 months compared to those who received non-sequential abemaciclib treatment, which resulted in a median PFS of 3.9 months.[93] Another 2023 multicentre, randomized controlled trial demonstrated clinical benefit after prior treatment with palbociclib plus ET but subsequent progression. Patients who continued palbociclib but switched ET had a clinical benefit rate of 34% and a median PFS of 2.6 months.[94] The MAINTAIN study demonstrated that patients with HR+/HER2− metastatic breast cancer who switched ET and got ribociclib saw a substantial increase in PFS compared to those who did not get ribociclib (5.29 vs 2.76 months, HR 0.57). This improvement was observed following prior treatment with CDK4/6 inhibitors and different forms of endocrine therapy.[95] Based on the PACE study, the addition of palbociclib to fulvestrant did not result in improved PFS compared to fulvestrant alone in patients with HR+/HER2− metastatic breast cancer who had previously received a combination of CDK4/6 inhibitor and endocrine therapy. The median PFS was 4.6 months with pepcid plus fulvestrant and 4.8 months with fulvestrant alone.[96] Currently, the combination of CDK4/6 inhibitors is still under investigation for post-progression treatment of ET, and replacement of other endocrine drugs in combination with CDK4/6 inhibitors could be one of the options after drug resistance.

New endocrine treatments

Selective oestrogen receptor degraders. In recent years, it has been revealed that targeting varying degrees of ER signalling is also a new technique. Researchers are now studying novel oral selective oestrogen receptor degraders (SERDs). These compounds attach to ER proteins found on the surface of cancer cells and trigger their disintegration through the cell's natural protein degradation processes. As a result, the levels of ER receptors decrease, which effectively inhibits the proliferation of cancer cells.[97] RAD1901 received Fast Track Designation from the FDA in 2018.[98] AZD9833,[99] GDC-9545[100] and SAR439859[101] are now developed and, along with RAD1901, have demonstrated efficacy against both normal and mutated ER-α breast cancer cell lines, as well as in models using patient-derived xenografts. They are currently undergoing phase III clinical studies. G1T48,[102] zn-c5,[103] D-050247[104] and zb716,[105] which are in early-stage trials, have also shown preclinical activity. Other phase II and III randomized trials of oral SERDs in combination with CDK4/6 inhibitors for the treatment of HR+/HER2-advanced breast cancer are still ongoing, including the EMBER-3, SERENA-6, SERENA-4, persevERA and AMEERA-5 trials.[106] Initial efficacy of the SERDs has been demonstrated, and it is believed that the future of the CDK4/6 inhibitor resistance problem will bring surprises.

Proteolysis targeting chimeras. Proteolysis targeting chimeras (PROTACs) are tiny compounds that have two different functional groups that can engage with E3 ubiquitin ligases (POIs) to facilitate the process of ubiquitination of POIs. Ubiquitinating enzymes break down them.[107] Due to the fact that PROTACs do not necessitate a target with high affinity or binding to the active site, they have the potential to target disease-associated proteins that are difficult to treat with small molecule inhibitors.[108] The first PROTAC ARV-471 is currently under clinical evaluation[109] and can degrade ER in ER+ breast cancer and inhibit the growth of ER-dependent cell lines through ER degradation.[110] Experiments have shown that more pronounced tumour growth inhibition (~130%) is observed when CDK4/6 inhibitors are combined with ARV-471, accompanied by a significant reduction in ER levels.[111] The above results support the continued development of ARV-471 as an ER PROTAC degrader.

Switch to other targeted drugs

Combination therapies targeting the PI3K/AKT/mTOR pathway. The PI3K/AKT/mTOR pathway is highly correlated with breast cancer, and over 40% of HR+/HER2− breast cancer patients had the PI3K3CA mutation.[112] The PI3K inhibitor alpelisib demonstrated its efficacy for the first time in the SOLAR-1 study, when alpelisib was added to fulvestrant therapy for patients with the PI3K3CA mutation, HR+, HER2− ABC patients improved the value of median OS by 7.9 months when it was added to fulvestrant,[113] and was approved for marketing by the FDA in 2019. The results of the BYLieve study demonstrated benefit for both alpelisib combined with ET after progression on CDK4/6 inhibitors, with

a more pronounced benefit in patients with a duration of prior CDK4/6 inhibitors ⩽6 months in the alpelisib combined with fulvestrant cohort.[114] After progression on CDK4/6 inhibitors, additional studies of apelalis are ongoing, including CAPTURE, EPIK-B5 and SEQUEI-Breast.[115] AKT serves as a connection or intermediary between the PI3K and mTOR signalling pathways. The TAKTIC research conducted efficacy evaluations on the AKT1 inhibitors ipatasertib and ET, as well as CDK4/6 inhibitors, following progression to CDK4/6 inhibitors. The results showed that after the failure of previous CDK4/6 inhibitor therapy, some patients (8/12) achieved good clinical outcomes with triple combination therapy and were well tolerated, and subsequent results remain to be seen.[116] In a separate randomized, double-blind experiment conducted in 2023, the effectiveness of the AKT inhibitor capivasertib, when used in combination with fulvestrant, was assessed for treating patients with advanced breast cancer that is HR+/HER2−. The results showed that the combination of capivasertib and fulvestrant medication significantly increased the PFS compared to treatment with fulvestrant alone. Specifically, the PFS was 7.3 months in the capivasertib combined with fulvestrant group, while it was only 3.1 months in the fulvestrant alone group. These findings were observed in patients whose disease had advanced despite prior treatment with or without a CDK4/6 inhibitor.[117] In the TRINITI research, the combination of exemestane, everolimus and ribociclib demonstrated a substantial improvement in PFS at 24 weeks. The complete response rate in the 95 patients who were eligible for efficacy assessment was 41.1%, surpassing the predetermined criterion of greater than 10% for the primary outcome.[118] Therefore, in the case of resistance to CDK4/6 inhibitors, directing efforts towards the PI3K/AKT/mTOR pathway shows potential.

ADC. Antibody-drug conjugate (ADC) is a humanized IgG-based monoclonal antibody that uses the antigen as a carrier and binds to cytotoxic reagents via ligands. The antibody component binds specifically to the target protein binding region (Fab) and then endocytosis of the ADC complex is achieved by endocytosis. Hydrolysis and/or acid cleavage of the ligand on the lysosomal membrane with proteins allows the release of the active ingredient from the tumour cells.[119] ADCs with cleavable linkers can also exert bystander-killing effects on neighbouring cells that may or may not express the target antigen.[120]

Trastuzumab deruxtecan (DS-8201) is an ADC medication that combines a humanized monoclonal antibody targeting the HER2 with a cytotoxic payload called DXd, which inhibits topoisomerase I. This medication has been authorized for the treatment of individuals with metastatic breast cancer that is HER2-positive.[121] DS-8201 efficiently targets tumour cells with low HER2 expression and delivers its powerful cytotoxic payload (drug–antibody ratio, 8:1) to nearby tumour cells that have varying levels of HER2 expression through a bystander effect.[122] The DESTINY-Breast04 clinical trial examined patients with metastatic breast cancer who had low levels of HER2 expression. These patients were divided into two groups: one receiving DS-8201 and the other receiving chemotherapy. The trial found that the median PFS was 9.9 months in the DS-8201 group and 5.1 months in the chemotherapy group (hazard ratio (HR), 0.50; $p < 0.001$). Additionally, the OS was 23.4 months in the DS-8201 group and 16.8 months in the chemotherapy group (HR, 0.64; $p = 0.001$).[123]

Sacituzumab govitecan (SG) is a novel ADC drug targeting the cell surface antigen Trop-2. The property of attaching the payload SN-38 to the antibody's linker allows SG to kill Trop-2-expressing tumour cells as well as neighbouring tumour cells.[124] The TROPiCS-02 phase III trial involved patients with advanced HR+/HER2− breast cancer who had previously been treated with a CDK4/6 inhibitor. These patients were randomly divided into two groups: the SG group and a group that received chemotherapy selected by their physician. The SG group showed a median PFS of 5.5 months (95% confidence interval (CI), 4.2–7.0), while the chemotherapy group had a median PFS of 4.0 months (95% CI, 3.1–4.4). The SG group demonstrated a statistically significant improvement in PFS compared to chemotherapy.[125]

Datopotamab deruxtecan (Dato-Dxd) is a newly developed ADC that consists of a topoisomerase I inhibitor payload and a monoclonal antibody targeting trophoblast surface antigen 2 (Trop-2).[126] Dato-Dxd has demonstrated initial effectiveness in a broad range of metastatic triple-negative breast cancer cases.[127] According to the TROPION-PanTumor01 trial, patients with advanced HR+/HER2− breast cancer or triple-negative breast cancer treated with Dato-Dxd had objective remission rates of 26.8% (95%

CI, 14.2–42.9) and 31.8% (95% CI, 18.6–47.6). The median duration of remission could not be assessed in the HR+/HER2− breast cancer group and was 16.8 months in the triple-negative breast cancer group. The median PFS for individuals diagnosed with HR+/HER2− breast cancer was 8.3 months, while for those with triple-negative breast cancer, it was 4.4 months. The efficacy and safety of Dato-DXd is now being assessed in a phase III clinical trial.[128] All of the above indicate that ADC has a clinical role after progression on CDK4/6 inhibitor therapy.

Poly (ADPribose) polymerase inhibitors. Poly (ADPribose) polymerase (PARP) inhibitors belong to a new category of focused medications that use synthetic lethal effects to target tumours with defective DNA damage repair and are the first targeted therapies capable of improving the prognosis of patients with hereditary tumours, revolutionizing germline BRCA1/2 (gBRCA1/2)-associated breast cancer treatment.[129] Pathogenic gBRCA1/2 gene mutations are present in approximately 5% of unscreened breast cancer patients. Patients carrying the gBRCA1 variant have predominantly triple-negative breast cancer, whereas patients carrying the gBRCA2 variant tend to develop HR+/HER2− breast cancer.[130] Results from the OlympiAD III phase I clinical study indicate that the PARP inhibitor olaparib demonstrated higher PFS and objective response rate (ORR), as well as a more favourable safety profile, when compared to standard therapy in patients with gBRCA1/2 mutant breast cancer who had previously had second-line chemotherapy.[131] Based on the findings of the EMBRACA research, talazoparib, a PARP inhibitor, demonstrated enhanced PFS of 8.6 months compared to 5.6 months, as well as an increased ORR of 62.6% compared to 27.2% in patients with the gBRCA1/2 mutation. However, it did not show any improvement in OS. Administering PARP inhibitors substantially enhances patients' quality of life and prolongs the duration of disease progression.[132,133] Despite these data from the pre-CDK4/6 inhibitor period, olaparib and talazoparib continue to be considered effective options for the treatment of patients with gBRCA1/2 mutant breast cancer after progression to endocrine-resistant CDK4/6 inhibitors following pretreatment with paclitaxel and anthracyclines, as both PARP inhibitors demonstrated sustained PFS benefit compared to chemotherapy.[134] Therefore, investigating PARP inhibitors is a valuable approach to consider for treating patients with breast cancer who have BRCA mutations.

HDAC inhibitors. HDAC inhibitors can induce anti-tumour effects by modulating the tumour microenvironment by changing the acetylation levels of histones and non-histone proteins. This leads to cell cycle arrest, differentiation and death in cancer cells.[135] Although HDAC inhibitors have been shown to display preclinical efficacy in the monotherapy of haematological and solid malignancies or combination with other anticancer agents, they are less effective as therapeutic agents for solid tumours alone. Therefore, considerable effort has been invested in evaluating rational combinations of HDAC inhibitors with other anticancer therapies in clinical trials.[136] The combination of HDAC inhibitors and ET is a highly promising strategy due to the ability of HDAC inhibitors to suppress the transcription of the ER. Acetylation plays a crucial role in the control of ER function. In a phase II clinical trial, 43 patients diagnosed with HR+/HER2− metastatic breast cancer that had worsened despite endocrine therapy were administered the HDAC inhibitor vorinostat along with tamoxifen. The trial revealed that 19% of the patients experienced a significant reduction in tumour size based on the Solid Tumour Response Evaluation Criteria, while 40% of the patients derived clinical benefits from the treatment.[137] Another HDAC inhibitor, tucidinostat, was demonstrated to significantly increase PFS (median, 9.6 months) in conjunction with exemestane (median, 3.8 months) compared to exemestane alone in the ACE trial, and so tucidinostat in combination with an aromatase inhibitor has been licensed in China.[38] Patients who had received CDK4/6 inhibitors were not included in the ACE study because CDK4/6 inhibitors were not available in China at the time the ACE study was conducted. Another clinical trial evaluated the efficacy and safety of tucidinostat in combination with endocrine therapy in HR+/HER2− breast cancer patients after progression on prior CDK4/6 inhibitors. The results showed that individuals treated with consecutive tucidinostat after failure of CDK4/6 inhibitor therapy had a mPFS of 4.5 months (95% CI: 4.2–4.8).[39] Indicating that the use of tucidinostat in combination with ET could be a potential sequential strategy.

Conversion to chemotherapy

Chemotherapy is still a viable treatment choice for patients who are receiving endocrine therapy alone or in combination with CDK4/6 inhibitors. It is usually used for quickly progressing or endocrine-refractory illness. Novel ET have been created to

postpone the transition to chemotherapy, but this approach is only successful in patients whose tumours continue to rely on ER.[115] Multiple retrospective studies have indicated that the PFS duration while transitioning to chemotherapy following the advancement of the disease after the first treatment with CDK4/6 inhibitors ranges from 7.2 to 9.7 months. In patients who did not respond to numerous courses of treatment with CDK4/6 inhibitors, the PFS was consistently 4–5.4 months and the median OS has not been determined.[138,139]

Conclusion

This research provides an overview of the mechanism behind resistance to CDK4/6 inhibitors in HR+/HER2− breast cancer and explores potential future therapeutic options. Currently, CDK4/6 inhibitors are extensively employed for treating HR+/HER2− breast cancer. Nevertheless, the issue of drug resistance has progressively emerged as a significant concern following prolonged usage. We studied numerous processes that contribute to CDK4/6 inhibitor resistance, including modifications in cell cycle regulatory pathways, reactivation of signal transduction pathways and changes in tumour microenvironment, which give a theoretical basis for devising new therapeutic options.

By targeting resistance pathways, researchers have begun to explore a variety of new therapeutic techniques. Among these, the introduction of new targeted medications, such as PI3K/AKT/mTOR pathway inhibitors and ADC therapies, provides fresh concepts and possibilities for the treatment of drug resistance. The concurrent use of HDAC inhibitors and PARP inhibitors exhibits promising synergistic effects that can impact the proliferation and viability of tumour cells via distinct routes. Furthermore, the latest iteration of endocrine therapy medications offers novel concepts and alternatives for addressing drug resistance in treatment.

In the future, HR+/HER2− breast cancer treatment will focus on customized treatment as a significant approach. By utilizing molecular profiling and pathological analysis, it becomes possible to more precisely anticipate how patients will respond to particular treatments, thus facilitating the practice of precision medicine. With the progress of technology and the growing knowledge of tumour biology, it is anticipated that an increasing number of treatment plans will be tailored to individual features to enhance patient outcomes and improve quality of life.

In summary, despite the obstacles posed by CDK4/6 inhibitor resistance, we are confident of additional breakthroughs in the treatment of HR+/HER2− breast cancer in the future by obtaining an understanding of the causes of resistance and implementing novel treatment techniques. Future research and clinical practice will focus on individualized therapy and multi-target combination techniques, which will offer new potential to enhance the survival rate and quality of life for patients.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

Sijia Wu: Data curation; Funding acquisition; Writing – original draft.

Junnan Xu: Formal analysis; Project administration.

Yiwen Ma: Formal analysis.

Guilian Liang: Methodology.

Jiaxing Wang: Project administration.

Tao Sun: Funding acquisition; Methodology; Writing – review & editing.

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ORCID iD

Sijia Wu https://orcid.org/0009-0003-8569-328X

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