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Review Article

Strategy of combining CDK4/6 inhibitors with other therapies and mechanisms of resistance

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Abstract: Cell cycle-dependent protein kinase 4/6 (CDK4/6) is a crucial kinase that regulates the cell cycle, essential for cell division and proliferation. Hence, combining CDK4/6 inhibitors with other anti-tumor drugs is a pivotal clinical strategy. This strategy can efficiently inhibit the growth and division of tumor cells, reduce the side effects, and improve the quality of life of patients by reducing the dosage of combined anticancer drugs. Furthermore, the combination therapy strategy of CDK4/6 inhibitors could ameliorate the drug resistance of combined drugs and overcome the CDK4/6 resistance caused by CDK4/6 inhibitors. Various tumor treatment strategies combined with CDK4/6 inhibitors have entered the clinical trial stage, demonstrating their substantial clinical potential. This study reviews the research progress of CDK4/6 inhibitors from 2018 to 2022, the related resistance mechanism of CDK4/6 inhibitors, and the strategy of combination medication.

Keywords: CDK4/6 inhibitor, resistance, epigenetic inhibitors

Introduction

Cell cycle-dependent kinases (CDKs) are a family of serine/threonine protein kinases that play a crucial role in regulating cell cycle progression. Among these, CDK4/6 has been extensively studied and is considered a key regulator of the cell cycle [1]. CDK4/6 regulates the cell cycle by controlling the activity of other proteins called cyclins. Cyclins are responsible for initiating various processes in the cell cycle, such as DNA replication and cell division. CDK4/6 activity requires its binding to Cyclin D to form a complex with kinase activity. This complex is responsible for driving the cell cycle from the quiescent G0 phase to the proliferative S phase by phosphorylating the retinoblastoma gene RB1 and activating the cell cycle [2]. Recent studies have highlighted the critical role of CDK4/6 in tumorigenesis and cancer development. CDK4/6 has been shown to promote tumor cell proliferation and metastasis, and its inhibition can effectively suppress tumor cell growth [3]. Moreover, CDK4/6 overexpression has been observed in several cancers, underscoring its use as a therapeutic target for cancer treatment [4]. Given the significance of CDK4/6 in cancer, it is imperative to develop novel anticancer drugs that target this protein. Targeting CDK4/6 has emerged as a promising approach for cancer therapy, and several CDK4/6 inhibitors have been developed and are currently being evaluated in clinical trials. Hence, CDK4/6 inhibition holds great promise as a therapeutic strategy for cancer treatment. Our research objective is to broaden the scope of CDK4/6 inhibitor application by presenting possible combination therapy strategies via comprehensive summary of CDK4/6 inhibitor resistance mechanisms.

The development of CDK4/6 inhibitors

CDK4/6 inhibitors represent a novel class of anticancer agents that specifically target the CDK4/6 protein. These inhibitors function by obstructing the formation of the CDK4/6-Cyclin D complex and its associated kinase activity, which is essential for the transition from G0 to S phase of the cell cycle [5]. By inhibiting

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CDK4/6 activity, these agents prevent tumor cell proliferation and division [6]. Recent ad vancements in the development and characterization of CDK4/6 inhibitors have yielded promising clinical results. Currently, four CDK4/6 inhibitors approved worldwide: Palbociclib (Pfizer), Ribociclib (Novartis), Abemaciclib (Lilly) and Dalpiciclib/SHR6390 (Jiangsu Hengrui) [7]. Palbociclib, the first FDA-approved small molecule tyrosine kinase inhibitor (TKI), is the most widely used CDK4/6 inhibitor [8]. Dalpiciclib/SHR6390 is the first Chinese novel highly selective CDK4/6 inhibitor approved by National Medical Products Administration (NMPA) for use in combination with fulvestrant (a selective estrogen receptor down-regulation (SERDs)), and suitable for hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2-) recurrent or metastatic breast cancer patients with disease progression after previous endocrine therapy [9].

Bone marrow suppression (e.g., neutropenia), gastrointestinal adverse reactions (e.g., diarrhea), abnormal liver function, prolonged QT interval, venous thromboembolism (VTE), and interstitial lung disease (ILD), are the six common adverse effects associated with the use of CDK4/6 inhibitors [10-12]. To minimize these side effects and improve therapeutic efficacy, the development of new CDK4/6 inhibitors is underway. Although CDK4/6 inhibitors are primarily utilized in the treatment of advanced HR+ breast cancer patients, their therapeutic effects on other cancers, such as triple-negative breast cancer (TNBC), are limited [13]. Therefore, current research on CDK4/6 inhibitors aims to expand their application to other cancer types.

Resistance mechanism of CDK4/6 inhibitor

CDK4/6 inhibitors have demonstrated efficacy in the treatment of advanced hormone receptor-positive (HR+) breast cancer patients [14]; however, the widespread use of CDK4/6 inhibitors has led to the emergence of acquired drug resistance in these patients. Furthermore, several breast cancer patients, including those with triple-negative breast cancer (TNBC), have shown resistance to CDK4/6 inhibitors [15]. Various molecular mechanisms have been identified as contributing to tumor cells' resistance to CDK4/6 inhibitors, with low tumor suppressor RB1 expression being the most common cause [16]. The reasons for the resistance mechanism of CDK4/6 inhibitors can be summarized as follows.

Low RB1 expression

RB1 is a tumor suppressor that plays a crucial role in regulating the cell cycle [17]. After phosphorylation of RB1 protein by CDK4/6-Cyclin D complex, the RB1 protein dissociates from the E2F transcription factor, which then activates transcription of downstream cell cycle-related genes and drives the cell cycle process [18]. However, RB1 gene mutations, transcriptional silencing, or hyperphosphorylation frequently occur in tumors, leading to decreased RB1 protein levels [19]. Low RB1 protein expression reduces the effectiveness of CDK4/6-Cyclin D complex inhibition, resulting in tumor cells' resistance to CDK4/6 inhibitors. A study using glioblastoma (GBM) xenograft cells found that the A193T missense mutation of exon 2 of Rb decreased RB1 protein, leading to its resistance to CDK4/6 inhibitors [20]. In addition, RB1 copy number changes and splicing mutations have been observed in several breast cancer cell lines, including T47D, LY2, and ZR-75-1. The transcription level of RB1 is low in MDA-MB-361 and ZR-75-1 cell lines, while the LY2 cell line has a complete deletion of the RB1 gene and protein [21]. These factors could reduce RB1 protein expression and contribute to resistance to CDK4/6 inhibitors. Palafox et al. have also demonstrated that point mutations in RB1 in estrogen receptor-positive (ER+) breast cancer can confer resistance to CDK4/6 inhibitors [22]. Thus, a detailed analysis of RB1 expression and regulation mechanisms could help to overcome CDK4/6 inhibitor resistance and broaden the scope of CDK4/6 inhibitors' application.

INK4 family protein overexpression

The INK4 protein family comprises p16 (encoded by CDKN2A), p15 (encoded by CDKN2B), p18 (encoded by CDKN2C), and p19 (encoded by CDKN2D), which are known to inhibit the activity of CDK4/6 proteins and regulate the cell cycle [23]. Specifically, p16 acts as an endogenous inhibitor of CDK4/6 kinase activity and competes with exogenous CDK4/6 inhibitors to bind CDK4/6, thereby reducing the sensitivity of tumor cells to CDK4/6 inhibitors [24].

Furthermore, the inhibitory effect of p16 on CDK4/6 is dependent on the function of RB1. However, p16 overexpression has been found to decrease CDK4, which in turn induces CDK4/6 inhibitor drug resistance [25]. Moreover, Green et al. have reported that the p16 protein family can impede the binding of CDK4/6 inhibitors to CDK4 [26]. Similarly, p15 and p18, which are also members of the INK4 family, have been shown to induce CDK6-mediated drug resistance. Reducing the expression of p15 and p18 or their binding to CDK6 can restore the sensitivity of CDK4/6 inhibitors [27]. Several studies have revealed that the expression of INK4 family members, particularly p16, significantly increases in various tumors [28], suggesting that the regulation of p16 protein expression levels may help overcome acquired drug resistance to CDK4/6 inhibitors.

Low CIP/KIP family protein expression

The CIP/KIP protein family, comprising p21 (encoded by CDKN1A), p27 (encoded by CDKN1B), and p57 (encoded by CDKN1C), plays a crucial role in inhibiting CDK kinase activity and stabilizing the CDK-Cyclin complex, thereby participating in cell cycle regulation during the G1/S phase transition [29]. The downregulation or functional impairment of CIP/KIP proteins has been associated with CDK4/6 inhibitor resistance. Studies have revealed that low p21 expression is one of the primary mechanisms underlying the acute resistance of breast cancer to CDK4/6 inhibitors [30]. Moreover, AbuHammad et al. have demonstrated that activating the tumor suppressor gene TP53 can induce p21 expression and restore sensitivity to CDK4/6 inhibitors. Therefore, comprehending the mechanisms underlying low CIP/KIP protein expression, especially p21, and TP53 activation in tumors, and identifying more effective strategies to promote CIP/KIP protein expression and activate TP53 can overcome CDK4/6 inhibitor resistance.

CDK6 amplification

CDK6, a direct substrate of CDK4/6 inhibitors, plays a critical role in promoting resistance to CDK4/6 inhibitors in preclinical breast cancer models [31]. Studies have shown that CDK6 upregulates p16 transcription in the presence of STAT3 and cyclin D [32]. Additionally, CDK6 and c-Jun, a transcription regulator and member of the leucine zipper family, synergistically upregulate vascular endothelial growth factor-A (VEGF-A), inducing angiogenesis, promoting cancer progression and drug resistance [33]. Xuewei et al. have reported that most tumor cells exhibit inherent resistance to CDK4/6 inhibitors owing to CDK6 expression, whereas tumors with low CDK6 expression rely on CDK4 function and are sensitive to CDK4/6 inhibitors. Furthermore, tumor cells expressing both CDK4 and CDK6 depend more on CDK6, thereby advancing the cell cycle. A lower ratio of CDK6 and CDK4 expression in tumors has been found to be more sensitive to CDK4/6 inhibitors [34]. Therefore, effective measures to inhibit CDK6 production in tumors or CDK6 that has already been produced can help overcome CDK4/6 inhibitor resistance.

CDK4 amplification

CDK4 is another substrate that is directly targeted by CDK4/6 inhibitors, and it plays a crucial role in the Cyclin D-CDK4/6-PRB pathway. CDK4 is widely expressed in several cancer types, and its overexpression has been identified as a potential biomarker for predicting resistance to conventional chemotherapy in patients with osteosarcoma [35]. A recent study investigating Rh 28 and Rh 41 cells of alveolar rhabdomyosarcoma (ARMS) has demonstrated that cells with CDK4 overexpression exhibit reduced CDK4/6 inhibitor activity, while glioma cells overexpressing CDK4 exhibit complete resistance to CDK4/6 inhibitors [20, 36]. Therefore, to overcome CDK4/6 inhibitor drug resistance, it is imperative to gain a better understanding of the underlying mechanisms involved in CDK4 overexpression in tumors and to develop more effective strategies for preventing CDK4 amplification.

Cyclin D1 overexpression

Cyclin D1 is a critical component of the CDK4/6 pathway and serves as a mitotic sensor by integrating extracellular mitotic signals with the cell cycle process. Cyclin D1 protein is frequently overexpressed in solid tumors, including breast cancer, especially estrogen receptor-positive (ER+) breast cancer, head and neck squamous cell carcinoma, pancreatic cancer, melanoma, endometrial carcinoma, colorectal cancer, and non-small cell lung cancer [37-42]. Cyclin D1

overexpression leads to increased CDK4/6 activity, resulting in abnormal downstream cell cycle regulation and aberrant cell proliferation [43]. While some studies have implicated CCND1 gene amplification, chromosomal rearrangement, or protein degradation damage as possible mechanisms of Cyclin D1 overexpression, the exact mechanism remains unclear [44]. Therefore, understanding the precise mechanism of Cyclin D1 overexpression in tumors and implementing more effective strategies to prevent Cyclin D1 amplification and reduce CDK4/6 activity may be crucial in overcoming drug resistance to CDK4/6 inhibitors.

CCNE1/2 or CDK2 amplification

Similar to the CDK4/6-Cyclin D complex, the complex formed by CDK2-cyclin E (encoded by CCNE1/2) plays a vital role in cell cycle progression from G0 to the S phase by phosphorylating RB1 [45]. Amplification of CCNE1/2 or CDK2 leads to increased occupancy of RB1 protein phosphorylation targets, thereby reducing the number of RB1 protein phosphorylation targets available for the CDK4/6-Cyclin D complex to bind. This phenomenon results in CDK4/6 inhibitor resistance [46]. Thus, inhibiting Cyclin E-CDK2 complex formation or preventing CCNE1/2 or CDK2 amplification may represent a viable strategy to overcome CDK4/6 inhibitor drug resistance.

E2F amplification

E2F is a transcription factor downstream of RB1, which governs the transcription of various cell cycle-related genes. During the cell cycle, the CDK4/6-Cyclin D complex phosphorylates RB1, resulting in the disruption of the RB1-E2F dimer, inactivating RB1, activating E2F transcription, and promoting entry into the S phase [47]. It has been established that E2F can upregulate AKT signal transduction through Gab2, leading to the reliance of cells on the AKT signaling pathway instead of the CDK4/6-RB1-E2F pathway, thereby conferring resistance to CDK4/6 inhibitors [48]. Therefore, a promising approach to enhance the efficacy of CDK4/6 inhibitors and overcome drug resistance would be to employ effective strategies to inhibit gene transcription or protein formation downstream of E2F while also inhibiting CDK4/6.

CDK7 amplification

CDK7 amplification is another mechanism of resistance to CDK4/6 inhibitors. CDK7 and CDK4/6 belong to the family of cell cycle-dependent kinases (CDKs). In addition to forming a kinase complex with Cyclin H, CDK7 functions as a transcription factor [49]. Studies have demonstrated that CDK7 overexpression imparts resistance to CDK4/6 inhibitors [50]. CDK7 has CDK-activated kinase (CAK) activity on CDK4 and CDK6, enhancing CDK4 and CDK6 phosphorylation. However, the precise mechanism of CDK7 in regulating cell cycle progression remains unclear, and further research is required to understand its potential role in G1 phase progression and to determine whether the combined use of CDK7 inhibitors can overcome resistance to CDK4/6 inhibitors.

WEE1 overexpression

WEE1 is a member of the serine/threonine protein kinase family, which phosphorylates Thr14 and Tyr15 of CDK1 and inhibits its kinase activity, thereby preventing cells from entering the division stage. WEE1 specifically regulates the progression from G2 to the M phase of the cell cycle [51]. Studies have identified that WEE1 and CDK1 synergistically inhibited DNA-damaged cells from entering mitosis [52]. Some studies have shown that inhibiting WEE1 can increase the sensitivity of breast cancer cells to CDK4/6 inhibitors, but the specific mechanism by which WEE1 overcomes CDK4/6 inhibitor resistance remains unclear [53]. Furthermore, the WEE1 inhibitor may collaborate with CDC25 phosphatase to inhibit triple-negative breast cancer (TNBC), which is resistant to CDK4/6 inhibitors [54].

MDM2 overexpression

MDM2 overexpression occurs in 20-30% of breast cancer patients and promotes ER+ breast cancer progression [55]. MDM2 can inhibit cell stability and senescence by negatively regulating TP53 [56]. Studies have shown that MDM2 inhibits the aging pathway in a TP53-dependent manner, thereby conferring resistance to CDK4/6 inhibitors [57]. Targeting MDM2 may provide a therapeutic option to overcome resistance to CDK4/6 inhibitors.

PTEN loss

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a protein tyrosine phosphatase (PTP) gene family member and a tumor suppressor gene that governs various biologic processes, including maintenance of genome stability, cell survival, migration, proliferation, and metabolism [58]. In breast cancer tumor biopsy samples, PTEN deletion in addition to low RB1 gene expression has been discovered in patients who are resistant to CDK4/6 inhibitors [59]. Bencivenga et al. confirmed that deletion of PTEN leads to CDK4/6 inhibitor resistance by activating AKT in vitro. This deletion-induced down-regulation of nuclear p27 resulted in resistance to CDK4/6 inhibitors [60]. Therefore, the development of effective strategies to inhibit PTEN deletion can enhance the effectiveness of CDK4/6 inhibitors.

FZR1 loss

FZR1 gene codes for CDH1, a regulatory subunit of the anaphase-promoting complex (APC), and plays an essential role in nerve development by regulating the cell cycle [61]. FZR1 is a co-activator of ubiquitin ligase APC/C and interacts with RB1 in the G1 phase of the cell cycle to ubiquitinate and inactivate RB1, thereby promoting uncontrolled cell cycle progression [62]. Ruijtenberg et al. demonstrated that FZR1, similar to RB1, could serve as a Cyclin D-CDK4/6 substrate, and its phosphorylation led to loss of APC/C activation [63]. The APC/C-FZR1 complex also degraded S-phase kinase-related protein 2 (SKP2), which inhibits p27, a natural inhibitor of CDK inhibitors [64]. Hence, further research is needed to understand the mechanism of FZR1 deletion and adopt strategies to overcome CDK4/6 inhibitor resistance caused by FZR1 deletion.

FAT1 loss

FAT atypical cadherin 1 (FAT1) is a protocadherin frequently mutated in human cancers, which results in tumor progression and can affect prognosis. FAT1 regulates various signaling pathways, including Wnt/β-catenin, Hippo, and MAPK/ERK pathways, through protein-protein interaction, thereby affecting cell proliferation, migration, and invasion [65]. Zhijiang et al. discovered that FAT1 deletion activated the Hippo pathway by inducing CDK6 expression, which led to CDK4/6 inhibitor resistance. Changes in YAP, MST1, LATS1, and NF2 also influenced CDK4/6 inhibitor resistance, but more research is necessary to confirm this finding [66]. Hence, the absence of FAT1 tumor suppressor is another promising avenue for overcoming CDK4/6 inhibitor resistance.

In summary, the decreased expression or amplification of numerous genes or proteins can impede the efficacy of CDK4/6 inhibitors. An exhaustive analysis of the affected genes and proteins may offer a means to overcome CDK4/6 inhibitor resistance. Furthermore, the activation of multiple pathways including fibroblast growth factor receptor (FGFR), PI3K/AKT/mTOR, EMT, and autophagy regulatory pathways contribute to CDK4/6 inhibitor resistance [67-70]. Consequently, the concurrent administration of inhibitors targeting multiple pathways may partially surmount drug resistance. Various studies have revealed that immunosuppression is also a key factor influencing CDK4/6 inhibitor resistance. Smruthi et al. identified that interferon-α and interferon-β pathways were enriched in breast cancer cells resistant to CDK4/6 inhibitors, implying that modulating immunosuppression could potentially overcome CDK4/6 inhibitor resistance [71].

Understanding the various mechanisms of resistance to CDK4/6 inhibitors is important for the development of effective treatment strategies for patients with advanced cancers. Identifying the factors that contribute to resistance can help to guide the selection of alternative therapies, such as combination therapies or higher dosage regimens of CDK4/6 inhibitors. Additionally, a deeper understanding of the mechanisms of resistance can inform the development of new drugs and treatment approaches that can overcome these mechanisms and improve patient outcomes. Ultimately, continued research into the various mechanisms of CDK4/6 inhibitor resistance is critical for advancing the field of cancer treatment and improving the lives of patients with advanced cancers.

Combined strategy of CDK4/6 inhibitors

Effective combination therapy involving CDK4/6 inhibitors has been shown to possess a synergistic effect, leading to a favorable antitumor

effect while reducing the side effects of other treatments. Overcoming drug resistance mechanisms of CDK4/6 inhibitors has become a prominent area of research in recent years. Despite the lack of a current effective remedy for low RB1 expression, the most fundamental cause of drug resistance, several drug combination strategies have demonstrated potential in improving the drug sensitivity of CDK4/6 inhibitors. Notably, combination therapy involving CDK4/6 inhibitors may include chemotherapy, endocrine therapy, targeted therapy, and immunotherapy. **Table 1** provides a comprehensive summary of the various combination therapy strategies for CDK4/6 inhibitors.

CDK4/6 inhibitor and chemotherapy

Chemotherapy is a conventional treatment approach for many cancer patients. However, its non-specific mechanism of action leads to the destruction of both cancerous and normal cells, resulting in significant toxic and side effects [72]. The primary chemotherapeutic drugs include alkylating agents (e.g., nitrogen mustard and carmustine), anthracyclines (e.g., adriamycin, epirubicin, and cyclophosphamide), platinum (e.g., carboplatin and cisplatin), taxanes (e.g., paclitaxel and docetaxel), and vinblastine (e.g., vincristine and vinorelbine).

Paclitaxel, a representative taxane, is a common chemotherapy drug used either alone or in combination with other drugs, typically carboplatin/cisplatin, to treat several solid tumors. Paclitaxel inhibits tumor cells by binding to tubulin and disrupting mitosis, and exhibits significant therapeutic effects in multiple cancer types [73]. Paclitaxel modulates tubulin during cell cycle division, while CDK4/6 inhibitors prevent RB1 phosphorylation during the G0 phase of the cell cycle. Thus, the combination of paclitaxel and CDK4/6 inhibitor jointly regulates the cell cycle. This combination therapy has been shown to decrease the side effects of paclitaxel therapy, such as hematopoietic stem cell death, and improve its therapeutic effect on triple-negative breast cancer cells [74, 75]. Additionally, CDK4/6 inhibitor and paclitaxel have a synergistic effect on K-Ras mutant lung adenocarcinoma cells by inducing apoptosis [76]. Recent studies have shown that the order of drug administration affects this synergistic effect, where paclitaxel and CDK4/6 inhibitors exhibit a more significant cytotoxic effect when administered first [77].

Platinum compounds are widely used in various cancer treatments. Patients with Human Papillomavirus (HPV)-negative head and neck squamous cell carcinoma (HNSCC) exhibited high sensitivity to CDK4/6 inhibitors, whereas those treated with cisplatin exhibited inherent resistance to CDK4/6 inhibitors. The sensitive mechanism was that CDK4/6 inhibitors inhibit CDK4, while the resistant mechanism was that cisplatin up-regulated c-Myc and Cyclin E expressions in DNA damage [78]. Acute renal injury (AKI) is a common disease caused by toxins, inflammation, or ischemic injury of renal tubular epithelial cells (RTECs), and is a side effect of cisplatin. Kim et al. revealed that CDK4/6 inhibitor could alleviate cisplatin-related AKI in an RB1-dependent manner, but the mechanism is unclear [79]. Liu et al. demonstrated that CDK4/6 inhibitor could reverse the acquired resistance of cisplatin in lung cancer cells by inhibiting cell proliferation and inducing apoptosis, providing a new therapeutic strategy for cisplatin-resistant lung cancer patients [80].

CDK4/6 inhibitor and endocrine therapy

Endocrine therapy is the primary treatment for ER-positive breast cancer. It contains a variety of drugs, such as selective estrogen receptor modulators (SERMs) (e.g., Tamoxifen and Raloxifene), selective estrogen receptor down-regulation (SERDs) (e.g., Fulvestrant), reversible aromatase inhibitors (e.g., Letrozole and Anastrozole), and irreversible steroidal aromatase inhibitor (e.g., Exemestane). Additionally, the recent introduction of proteolytic targeting chimera (PROTAC), a heterobifunctional molecule consisting of an estrogen receptor ligand, and another ligand as the substrate of E3 ubiquitin ligase complex, has provided a novel approach to endocrine therapy [81]. Despite the benefits of these therapies, drug resistance remains a significant issue, which has led to the investigation of CDK4/6 inhibitors for overcoming this problem.

Recent studies have shown that high mobility group box 1 (HMGB1) is associated with shorter progression-free survival (PFS) after ER-positive breast cancer surgery. HMGB1 promotes tamoxifen resistance by activating the TLR4-NF-κB pathway, and when combined with a CDK4/6 inhibitor, it reverses tamoxifen resistance by inhibiting HMGB1 expression [82]. The PALOMA-2 clinical trial has demonstrated that the combination of letrozole and CDK4/6 inhibi-

Table 1. Progress in combination therapy strategies of CDK4/6 inhibitors

Name of CDK4/6 inhibitor	Combined inhibitor type	Inhibitor name	Combined effect/mechanism	Cancer species	References
CDK4/6 inhibitor and chemotherapy					
Palbociclib/G1T28 (Trilaciclib)/LY2835219	Microtubule protein inhibitor	Paclitaxel	Reduce the death of hematopoietic stem cells; Induce apoptosis; Enhance the efficacy of chemotherapy	ER+ breast cancer, TNBC, lung cancer	[74-77]
Ribociclib/PD-0332991	-	Cisplatin	Relieve kidney injury; Block proliferation; Induce apoptosis; Reverse chemotherapy resistance	Non-small cell lung cancer	[78-80]
CDK4/6 inhibitor and endocrine therapy					
Abemaciclib	Selective estrogen receptor modulators (SERMs)	Tamoxifen	Inhibition of HMGB1 reversed drug resistance in endocrine therapy	ER+ breast cancer	[82]
Palbociclib	Selective estrogen receptor down-regulation (SERDs)	Fulvestrant	Prolong OS	Advanced breast cancer	[84-86]
Palbociclib	Aromatase inhibitor (AI)	Letrozole	Prolong PFS	ER+/HER2- breast cancer	[83]
CDK4/6 inhibitor and targeted therapy					
CDK4/6 inhibitor +PARP inhibitor					
Palbociclib/Abemaciclib	PARP inhibitor	Olaparib	Inhibit differentiation, induce apoptosis, and block proliferation	Prostatic cancer	[90]
Ribociclib (LEE011)/Palbociclib (PD0332991)		Olaparib	Inhibit PARP1 expression and damage DNA damage repair	Lung cancer	[91]
Palbociclib		Olaparib	Overcoming drug resistance of PARP inhibitors	TNBC	[92]
CDK4/6 inhibitor and other kinase inhibitors					
Abemaciclib	EGFR inhibitor	Osimertinib	Prevent EGFR inhibitor resistance	Non-small cell lung cancer	[94]
Palbociclib		Osimertinib	Inhibit RB1 phosphorylation, block proliferation, and overcome EGFR inhibitor resistance		[95]
SHR6390	EGFR and HER-2 dual inhibitors	Pyrotinib	Overcome the drug resistance of EGFR and HER-2 double inhibitors	Gastric cancer	[96]
		Pyrotinib	Block proliferation, migration, and invasion, and prolong recurrence time	Breast cancer	[97]
Abemaciclib	PI3K inhibitor	Alpelisib	Suppress tumor progression	HER2+ breast cancer	[98]
Palbociclib	AKT inhibitor	Capivasertib	Inhibit tumor progression and metastasis		[99]
Ribociclib	CK1ε	D4476	Down-regulate CDK6 expression and overcome CDK4/6 inhibitor resistance	ER+/HER2- breast cancer	[100]
Ribociclib	WEE1 inhibitor	AZD1775/Adavosertib	Block proliferation, induce apoptosis, and overcome drug resistance of CDK4/6 inhibitors	ER+ breast cancer	[53]
Palbociclib	TTK inhibitor	CFI-402257	Overcome drug resistance of CDK4/6 inhibitors	ER+ breast cancer	[21]
	Aurora kinase A/B inhibitor	Aliserlib/Baraserlib			

CDK4/6 inhibitor and epigenetic inhibitor					
Palbociclib	DNMT target	-	Inhibit p16, inhibit tumor progression	Lung cancer	[104]
Palbociclib	EZH2 target	AC1Q3QWB	Arrest proliferation	glioblastoma	[107, 108]
-	PRMT target	-	Prevent CDK4/6 from forming a complex with CyclinD; Arrest proliferation	-	[110]
Palbociclib	HDAC target	entinostat/(MS-275)	Activation of p21 inhibits tumor progression	ER+ breast cancer	[112, 113]
Palbociclib	BET inhibitor	JQ1	Prevent proliferation and promote aging	TNBC	[115]
Palbociclib	KDM inhibitor	GSK-J4	Decrease the expression of the E2F target gene and the chromatin accessibility of MYCN	Neuroblastoma	[117]
CDK4/6 inhibitor and other target inhibitors					
Palbociclib	lysosome	Antibiotic azithromycin, antidepressant siramesine, antimalaria compound chloroquine	Enhance the synthesis of lysosomes, increase the number of lysosomes, and overcome the drug resistance of CDK4/6 inhibitors	TNBC	[119]
-	PROTAC	-	Degradation of CDK4/6	-	[120, 121]
CDK4/6 inhibitor and immunotherapy					
Palbociclib/G1T28 (trilaciclib)/Abemaciclib	Immune checkpoint inhibitor	PD-1	Improve immune microenvironment; Enhance anti-tumor immunity; Improve T cell memory; Sensitizing the therapeutic effect of PD-1	Colorectal cancer, ER+ breast cancer	[126, 127]

tor prolongs PFS by 13.1 months (14.5 months vs. 27.6 months) compared to letrozole alone [83]. In metastatic ER-positive breast cancer, ESR1 mutation is frequently observed during aromatase inhibitor treatment, but is rare in the primary tumor [84]. Patients with ESR1 mutation benefit from the combination of fulvestrant and CDK4/6 inhibitor [85]. The clinical trial of advanced breast cancer has shown that fulvestrant combined with CDK4/6 inhibitor prolongs overall survival (OS) by 5.2 months (29.7 months vs. 34.9 months) compared to fulvestrant alone [86].

CDK4/6 inhibitor and targeted therapy

Targeted therapy is a treatment method that targets a specific cancer site, which could be a gene or a protein molecule in tumor cells. Targeted therapy interferes with tumor metastasis, and inhibits tumor cell proliferation, differentiation, and apoptosis [87]. Commonly used targeted therapies included several inhibitor types targeting PARP, angiogenesis, kinase, phosphatase, and epigenetic modulation [88]. Compared to traditional chemotherapy, targeted therapy has minimal side effects and therefore become increasingly popular among patients.

CDK4/6 inhibitor and PARP inhibitor

Poly ADP-ribose Polymerase (PARP) is a crucial enzyme involved in the repair of single-stranded DNA gaps. PARP inhibitors are small molecule drugs that exhibit a synergistic lethal effect with BRCA1/BRCA. Currently, Olaparib, Rucaparib, Niraparib, Talazoparib (Talzenna), and Veliparib (ABT-888) have been approved by the FDA for cancer treatment [89].

It has been discovered that combining PARP inhibitor (Olaparib) and CDK4/6 inhibitor (Palbociclib/Abemaciclib) therapy can synergistically inhibit the RB1-E2F1 signal axis at the transcription and post-translation levels, resulting in cell cycle arrest and E2F1 gene target inhibition. Moreover, combined therapy can suppress tumor cell growth, induce cell apoptosis, and inhibit neuroendocrine differentiation [90]. Additionally, CDK4/6 inhibitor-induced E2F1-RB1-HDAC1-PRC2/EZH2 inhibitory complex formation can down-regulate transcription and inhibit PARP1 expression, thereby impairing DNA repair mediated by OGG1 and improving lung cancer cell sensitivity to topoisomerase II (Etoposide) inhibition [91]. Zhu et al. have demonstrated that β-catenin overexpression, particularly the over-phosphorylation of its Ser675 site, activates the Wnt signaling pathway, which mediates the resistance of PARP inhibitor (Olaparib), whereas CDK4/6 inhibitor (Palbociclib) can significantly inhibit this resistance [92]. It has been proven that PARP inhibitor (Olaparib) and CDK4/6 inhibitor (Palbociclib) exhibit a synergistic effect in triple-negative breast cancer (TNBC) with BRCA mutation by significantly inhibiting homologous recombination repair (HR), increasing DNA damage, and inhibiting tumor progression.

CDK4/6 inhibitor and other kinase inhibitors

Small-molecule kinase inhibitors have emerged as a promising therapeutic target in recent decades, providing a wide range of treatment options. Among the popular kinase inhibitors are the cyclin-dependent kinase (CDK) inhibitor, epidermal growth factor receptor (EGFR) inhibitor, fibroblast growth factor receptor (FGFR) inhibitor, vascular endothelial growth factor receptor (VEGFR) inhibitor, Bruton tyrosine kinase (BTK) inhibitor, Janus kinases (JAK) inhibitor, and phosphatidylinositol 3-kinase (PI3K) inhibitor [93]. FDA has approved numerous kinase inhibitors for the treatment of various types of cancers.

CDK4/6 inhibitors and EGFRs inhibitors: EGFRs represent a promising therapeutic target for non-small cell lung cancer. The EGFR tyrosine kinase inhibitor (TKI) has advanced to the fourth generation. In the context of EGFR inhibitor (Osimertinib) resistance, the non-small cell lung cancer (NSCLC) cell model upregulated RB1 protein phosphorylation levels and demonstrated sensitivity to CDK4/6 inhibition. Notably, co-treatment with the EGFR inhibitor (Osimertinib) and CDK4/6 inhibitor (Abemaciclib) did not exhibit a synergistic effect in inhibiting cell growth, spheroid formation, colony formation, or induced aging. However, the use of CDK4/6 inhibitor (Abemaciclib) effectively prevented the emergence of EGFR inhibitor (Osimertinib) drug resistance [94]. In their study, Qin et al. demonstrated the efficacy of the CDK4/6 inhibitor palbociclib in overcoming acquired resistance to the EGFR inhibitor Osimertinib in non-small cell lung cancer (NSCLC).

The combined treatment of these inhibitors exhibited a synergistic effect by arresting cell cycle progression and inhibiting tumor growth. This effect was achieved by significantly reducing the phosphorylation level of the retinoblastoma protein (RB1) through the inhibition of CDK4/6 function, which blocked cell proliferation [95]. The imbalance in the CCND1-CDK4/6-RB1 axis is implicated in the development of resistance to the dual EGFR/HER2 inhibitor Pyrotinib in HER2+ metastatic gastric cancer. However, the CDK4/6 inhibitor SHR6390 has been demonstrated to overcome this resistance and has shown efficacy in clinical patients [96]. In HER2+/ER+ breast cancer patients, the combination of ubiquitin kinase inhibitor Pyrotinib and CDK4/6 inhibitor SHR6390 had a synergistic effect in blocking cell cycle progression, cancer cell proliferation, migration, and invasion. Moreover, the combination of these drugs prolonged tumor recurrence time in a xenograft tumor model [97]. The use of CDK4/6 inhibitors has great potential in overcoming EGFR inhibitors, thereby expanding the potential applications for EGFR inhibitors in the treatment of cancer.

CDK4/6 inhibitor and other kinase inhibitors: CDK4/6, a downstream target of the PI3K/AKT/mTOR signaling pathway, is frequently activated in breast cancer, accounting for 30-40% of cases. Aberrant activation of the PI3K/AKT/mTOR pathway contributes to CDK4/6 inhibitor resistance [68]. Recent studies have indicated that PI3K inhibitors can reduce Cyclin D1 expression, thereby inhibiting the downstream CDK4/6 signaling pathway. In preclinical models sensitive to CDK4/6 inhibitors, combined inhibition of CDK4/6 and PI3K completely regressed tumors, which was superior to single drug treatment [98]. The combination of CDK4/6 inhibitors and endocrine therapy is effective in treating advanced ER+ breast cancer. The triple combination of endocrine therapy (Fulvestrant), CDK4/6 inhibitor (Palbociclib), and AKT inhibitor (Capivasertib) continuously inhibits breast cancer cell growth and metastasis, as well as xenograft tumor progression. This effect is mediated through the simultaneous targeting of the cell cycle pathway and PI3K/AKT/mTOR pathway [99]. CDK4/6 inhibitors can induce drug resistance in breast cancer by promoting ubiquitination and proteasome degradation of RB1, as well as transcriptional activation of CDK6. Inhibition of Casein kinase-1-ε (CK1ε) can prevent RB1 degradation and downregulate CDK6 expression, thereby improving the efficacy of CDK4/6 inhibitors. CK1ε has been identified as a promising target to overcome CDK4/6 inhibitor resistance [100]. Approximately 20% of tumors exhibit intrinsic resistance to CDK4/6 inhibitors. WEE1 inhibitors (AZD1775/Adavosertib) have been shown to overcome this resistance by inducing apoptosis via increasing G2/M phase arrest [53]. Recent studies have identified the spindle assembly checkpoint as a therapeutic target for CDK4/6 inhibitor-resistant ER+ breast cancer, with TTK inhibitors (CFI-402257) and Aurora kinase A/B (Aliserlib/Baraserlib) showing high sensitivity in CDK4/6 drug-resistant models [21].

CDK4/6 inhibitor and epigenetic inhibitor

Epigenetic inhibitors are a class of therapeutic agents that modulate gene expression patterns without altering the DNA sequence. Small-molecule inhibitors have emerged as the primary strategy for achieving targeted epigenetic regulation, specifically by regulating the "writing", "reading", and "erasing" of epigenetic marks. Examples of representative epigenetic targets and drugs that have advanced to clinical trials include DNA methyltransferase (DNMT) inhibitors such as Azacitidine, Decitabine, and Guadecitabine; histone deacetylase (HDAC) inhibitors such as Entinostat and Vorinostat; histone methyltransferase (HMT) inhibitors such as Enhancer of Zeste 2 (EZH2) inhibitors, Protein arginine methyltransferase (PRMT) inhibitors, G9a/GLP inhibitors, DOT1L inhibitors, SMYD2/3 inhibitors, and SETD7/8 inhibitors; histone demethyltransferase (HDM) inhibitors such as LSD1 inhibitors; and domain protein inhibitors such as BET protein domain inhibitors, PHD protein domain inhibitors, CBX protein domain inhibitors, Tudor protein domain inhibitors, and MBT family protein domain inhibitors [101].

Combined with DNMT inhibitor: The DNMT family of enzymes is responsible for catalyzing DNA methylation, whereby a methyl group is covalently added to the cytosine of a CpG dinucleotide. In eukaryotes, there exist three types of DNMTs, namely DNMT1, DNMT3a, and DNMT3b, which play critical roles in maintaining

genomic stability and regulating various cellular processes including cell cycle, apoptosis, and embryonic development [102]. DNMT inhibitors have been shown to re-express genes silenced by DNA methylation, leading to direct antitumor effects such as cell cycle arrest, apoptosis, and differentiation. Prominent DNMT inhibitors that have entered clinical trials include Azacitidine, Decitabine, and Guadecitabine [103]. Notably, Li et al. have demonstrated that increased methylation of the p16 gene enhances the sensitivity of lung and gastric cancer cells to CDK4/6 inhibitor palbociclib [104].

Combined with an EZH2 inhibitor: EZH2 is a catalytic subunit of the polycomb repressive complex 2 (PRC2), which is involved in the epigenetic regulation of gene expression. As a highly conserved histone methyltransferase, EZH2 specifically catalyzes the monomethylation, dimethylation, and trimethylation of lysine 27 on histone H3 [105]. It has been identified as a novel target for cancer treatment due to its role in promoting tumor growth and metastasis. Recent studies have demonstrated that CDK4/6 phosphorylates EZH2, leading to the activation of the STAT3 pathway. The CDK4/6-EZH2 pathway has been shown to be a potential therapeutic target for psoriasis, and treatment with CDK4/6 inhibitors or EZH2 inhibitors has been found to benefit psoriasis patients [106]. In addition, AC1Q3QWB has been found to block the recruitment of PRC2 and increase the expression of tumor suppressors by interfering with the interaction between HOTAIR and EZH2 [107]. Interestingly, the combination of AC1Q3QWB and CDK4/6 inhibitor (Palbociclib) has been found to have a more significant cell cycle retardation effect than CDK4/6 inhibitor (Palbociclib) alone in gliomas with high HOTAIR and EZH2 expressions but low CWF19L1 expression. These findings suggest that the use of EZH2 inhibitors in combination with other targeted therapies may have therapeutic benefits for various types of cancer [108].

Combined with PRMT inhibitor: PRMTs are enzymes that catalyze the methylation of various proteins, including both histones and non-histones. There are nine members of the PRMT family, designated PRMT1-9 [109]. Currently, most of the small molecule PRMT inhibitors that are entering clinical trials are PRMT5 inhibitors. PRMT1, for example, methylates CDK4/6, which prevents CDK4/6 from forming a complex with CyclinD. In addition, PRMTs methylate cyclin kinase inhibitors (CKIs), such as p16, p21, and p27. Furthermore, PRMT1 and PRMT5 methylate E2F to inhibit the transition of the cell cycle from G0 to S phase [110]. Thus, combining a PRMT inhibitor and a CDK4/6 inhibitor could potentially synergistically inhibit cell cycle progression, although further research is needed to confirm this hypothesis.

Combined with HDAC inhibitor: Histone deacetylases (HDACs) consist of 18 isoforms that can achieve global deacetylation. Currently, four HDAC inhibitors have been approved by the FDA, and one of them, tucidinostat, developed by Microchip, is also approved in China [111]. HDAC1, as a reversible regulator of cell proliferation, can inhibit p21 expression. On the other side, p21 gene deletion rescues HDAC1 function [112]. Although HDAC's involvement in the study of the CDK4/6 inhibitors resistance mechanism has been limited, several studies have confirmed that inhibiting HDAC can enhance the therapeutic effect of CDK4/6 inhibitors by activating p21. In ER+ breast cancer, the combination of the HDAC inhibitor entinostat (MS-275) and CDK4/6 inhibitor palbociclib can synergistically block cell cycle progression [113].

Combined with BET inhibitor: Brominedomain and terminal outer domain (BET) family proteins, which include BRD2, BRD3, BRD4, and BRDT, are involved in the regulation of transcription, cell cycle progression, and cell differentiation [114]. Of these, BRD4 has been most extensively studied. Ge et al. recently demonstrated that combining a CDK4/6 inhibitor (Palbociclib), a tubulin inhibitor (Paclitaxel), and a BET inhibitor (JQ1) can synergistically induce cell cycle arrest and promote tumor cell senescence [115].

Combined with KDM inhibitor: KDM6, a member of the lysine demethylated protein family, includes KDM6A, KDM6B, and UTY. KDM inhibitors demethylate by targeting histone lysine residues [116]. Previous studies have shown that KDM6B promotes CDK4/6-pRB-E2F activation in MYCN-amplified neuroblastoma by stabilizing the enhancer. Inhibition of KDM6B resulted in decreased expression of E2F target genes and chromatin accessibility of MYCN, as

well as an increase in the inhibitory marker H3K27me3 and a decrease in the active marker H3K4me1. Overexpression of CDK4/6 or knockout of RB1 led to resistance to the KDM6 inhibitor (GSK-J4) [117]. Therefore, KDM6B may serve as an effective therapeutic target to overcome resistance to CDK4/6 inhibitors.

In addition, research on the combination of CDK4/6 and epigenetic inhibitors gained attraction with the advancement of epigenetic research. Recent studies suggest that crosstalk between CDK4/6 and histone methyltransferase SMYD2 regulates gene transcription, tubulin methylation, and cilium generation. CDK4/6 positively regulates the phosphorylation and enzyme activity of SMYD2, while SMYD2 also positively regulates CDK4/6 expression [118]. Multiple strategies that combine CDK4/6 inhibitors with epigenetic inhibitors are currently recruiting patients for clinical trials.

CDK4/6 inhibitor and other targets

Combined with lysosomotropic or lysosomal-destroying compound: Recent studies have demonstrated that several triple-negative breast cancer (TNBC) cells heavily rely on CDK4/6 for their proliferation. However, these TNBC cells can develop resistance to CDK4/6 inhibitors due to the absorption of the inhibitor by cancer lysosomes. In fact, lysosomal enhancement and upregulation in TNBC cells could facilitate CDK4/6 inhibitor absorption and increase drug resistance. To overcome this challenge, co-administration of lysosomal-destabilizing agents such as the antibiotic azithromycin, antidepressant siramesine, or antimalarial compound chloroquine has been shown to render drug-resistant TNBC cells sensitive to CDK4/6 inhibitors [119].

PROTAC-mediated blocking of CDK4/6: In addition to the conventional CDK4/6 inhibitors, proteolytic targeting chimera (PROTAC) technologies have also been developed as a promising strategy for CDK4/6 inhibition. PROTACs are heterobifunctional molecules comprising a receptor ligand and a substrate ligand for the E3 ubiquitin ligase complex, which recruits the target protein to the E3 ubiquitin ligase for ubiquitination and degradation [120]. Various CDK4/6-targeted PROTACs have been designed to directly degrade CDK4/6 [121], thereby overcoming the drug resistance of CDK4/6 inhibitors.

CDK4/6 inhibitor and immunotherapy

Tumor immunotherapy is a treatment modality that harnesses the immune system's ability to recognize and eliminate malignant cells, thereby providing an enhanced immune response to the body [122]. It involves various strategies, including monoclonal antibodies, cancer vaccines, adoptive cell therapy, oncolytic viruses, immune checkpoint inhibitors, cytokines, and immune adjuvants. CAR-T cell therapy and immune checkpoint inhibitors are among the most effective modalities in clinical practice [123].

Recent studies have indicated that CDK4/6 inhibitors modulate the tumor immune microenvironment, facilitating cytotoxic T cell-mediated tumor inhibition and enhancing anti-tumor immunity [124, 125]. CDK4/6 inhibitors can also boost T cell immune memory, thereby further enhancing anti-tumor immunity [126]. Furthermore, CDK4/6 inhibitors have been found to enhance the response to immune checkpoint inhibitors (PD-1) [127]. Although several studies have identified CDK4/6 inhibitors as regulators of immunity, further research is required to fully understand the impact of CDK4/6 on immune regulation. Nonetheless, it is possible that regulating immunosuppression could be a promising therapeutic avenue for overcoming CDK4/6 inhibitors, but more extensive research is necessary.

In summary, combination therapies involving CDK4/6 inhibitors have proven to be a promising strategy for improving treatment outcomes in patients with hormone receptor-positive breast cancer. By targeting multiple pathways, these therapies can enhance the effectiveness of CDK4/6 inhibitors and overcome treatment resistance. The use of CDK4/6 inhibitors in combination with PI3K inhibitors, immune checkpoint inhibitors, endocrine therapy, or chemotherapy has all shown promising results in clinical trials, with improved progression-free survival and overall survival rates observed. These findings highlight the potential for collaboration between different drugs and treatment modalities to optimize cancer treatment and improve patient outcomes.

Conclusions

Due to the clinical efficacy displayed by various CDK4/6 inhibitors, research in this field remains active. Researchers aim to enhance the efficacy of existing CDK4/6 inhibitors and develop novel ones to cater to the needs of a larger patient population. Moreover, investigations into the mechanisms of drug resistance to CDK4/6 inhibitors are advancing, and it is believed that more combined strategies will emerge in the future to overcome such resistance. Furthermore, the combination of CDK4/6 inhibitors and immunotherapy shows great potential for development in sensitized immunotherapy, and further exploration in this direction is warranted.

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Disclosure of conflict of interest

None.

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References

[1] Cicenas J and Valius M. The CDK inhibitors in cancer research and therapy. J Cancer Res Clin Oncol 2011; 137: 1409-18.
[2] VanArsdale T, Boshoff C, Arndt KT and Abraham RT. Molecular pathways: targeting the cyclin D-CDK4/6 axis for cancer treatment. Clin Cancer Res 2015; 21: 2905-10.
[3] Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, Zamboni WC, Wong KK, Perou CM and Sharpless NE. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. J Natl Cancer Inst 2012; 104: 476-87.
[4] Goel S, Bergholz JS and Zhao JJ. Targeting CDK4 and CDK6 in cancer. Nat Rev Cancer 2022; 22: 356-72.
[5] Fassl A, Geng Y and Sicinski P. CDK4 and CDK6 kinases: from basic science to cancer therapy. Science 2022; 375: eabc1495.
[6] Sherr CJ, Beach D and Shapiro GI. Targeting CDK4 and CDK6: from discovery to therapy. Cancer Discov 2016; 6: 353-67.
[7] O'Leary B, Finn RS and Turner NC. Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol 2016; 13: 417-30.
[8] Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW and Jager A. Inhibiting CDK4/6 in breast cancer with palbociclib, ribociclib, and abemaciclib: similarities and differences. Drugs 2021; 81: 317-31.
[9] Liu YP, Hu MH, Lin PP, Li T, Liu SQ, Wang YY, Li SR, Li XK, Wang CJ and Cao Y. Evaluation of the effect of food on the pharmacokinetics of SHR6390, an oral CDK4/6 inhibitor, in healthy volunteers. Drugs R D 2022; 22: 175-82.
[10] Rugo HS, Huober J, García-Sáenz JA, Masuda N, Sohn JH, Andre VAM, Barriga S, Cox J and Goetz M. Management of abemaciclib-associated adverse events in patients with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: safety analysis of MONARCH 2 and MONARCH 3. Oncologist 2021; 26: e53-65.
[11] Diéras V, Harbeck N, Joy AA, Gelmon K, Ettl J, Verma S, Lu DR, Gauthier E, Schnell P, Mori A, Rugo HS and Finn RS. Palbociclib with letrozole in postmenopausal women with ER+/HER2- advanced breast cancer: hematologic safety analysis of the randomized PALOMA-2 trial. Oncologist 2019; 24: 1514-25.
[12] Durairaj C, Ruiz-Garcia A, Gauthier ER, Huang X, Lu DR, Hoffman JT, Finn RS, Joy AA, Ettl J, Rugo HS, Zheng J, Wilner KD and Wang DD. Palbociclib has no clinically relevant effect on the QTc interval in patients with advanced breast cancer. Anticancer Drugs 2018; 29: 271-80.
[13] Sánchez-Martínez C, Lallena MJ, Sanfeliciano SG and de Dios A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs: recent advances (2015-2019). Bioorg Med Chem Lett 2019; 29: 126637.
[14] Asghar U, Witkiewicz AK, Turner NC and Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 2015; 14: 130-146.
[15] Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, Pearson A, Guzman M, Rodriguez O, Grueso J, Bellet M, Cortés J, Elliott R, Pancholi S, Baselga J, Dowsett M, Martin LA, Turner NC and Serra V. Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. Cancer Res 2016; 76: 2301-2313.
[16] Guarducci C, Bonechi M, Boccalini G, Benelli M, Risi E, Di Leo A, Malorni L and Migliaccio I. Mechanisms of resistance to CDK4/6 inhibitors in breast cancer and potential biomarkers of response. Breast Care (Basel) 2017; 12: 304-8.

[17] Dyson NJ. RB1: a prototype tumor suppressor and an enigma. Genes Dev 2016; 30: 1492-502.
[18] Classon M and Harlow E. The retinoblastoma tumour suppressor in development and cancer. Nat Rev Cancer 2002; 2: 910-7.
[19] Sharma A, Comstock CE, Knudsen ES, Cao KH, Hess-Wilson JK, Morey LM, Barrera J and Knudsen KE. Retinoblastoma tumor suppressor status is a critical determinant of therapeutic response in prostate cancer cells. Cancer Res 2007; 67: 6192-6203.
[20] Cen L, Carlson BL, Schroeder MA, Ostrem JL, Kitange GJ, Mladek AC, Fink SR, Decker PA, Wu W, Kim JS, Waldman T, Jenkins RB and Sarkaria JN. p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells. Neuro Oncol 2012; 14: 870-81.
[21] Soria-Bretones I, Thu KL, Silvester J, Cruickshank J, El Ghamrasni S, Ba-Alawi W, Fletcher GC, Kiarash R, Elliott MJ, Chalmers JJ, Elia AC, Cheng A, Rose AAN, Bray MR, Haibe-Kains B, Mak TW and Cescon DW. The spindle assembly checkpoint is a therapeutic vulnerability of CDK4/6 inhibitor-resistant ER+ breast cancer with mitotic aberrations. Sci Adv 2022; 8: eabq4293.
[22] Palafox M, Monserrat L, Bellet M, Villacampa G, Gonzalez-Perez A, Oliveira M, Brasó-Maristany F, Ibrahimi N, Kannan S, Mina L, Herrera-Abreu MT, Òdena A, Sánchez-Guixé M, Capelán M, Azaro A, Bruna A, Rodríguez O, Guzmán M, Grueso J, Viaplana C, Hernández J, Su F, Lin K, Clarke RB, Caldas C, Arribas J, Michiels S, García-Sanz A, Turner NC, Prat A, Nuciforo P, Dienstmann R, Verma CS, Lopez-Bigas N, Scaltriti M, Arnedos M, Saura C and Serra V. High p16 expression and heterozygous RB1 loss are biomarkers for CDK4/6 inhibitor resistance in ER+ breast cancer. Nat Commun 2022; 13: 5258.
[23] LaPak KM and Burd CE. The molecular balancing act of p16(INK4a) in cancer and aging. Mol Cancer Res 2014; 12: 167-83.
[24] Witkiewicz AK, Knudsen KE, Dicker AP and Knudsen ES. The meaning of p16(ink4a) expression in tumors: functional significance, clinical associations and future developments. Cell Cycle 2011; 10: 2497-503.
[25] Medema RH, Herrera RE, Lam F and Weinberg RA. Growth suppression by p16ink4 requires functional retinoblastoma protein. Proc Natl Acad Sci U S A 1995; 92: 6289-93.
[26] Green JL, Okerberg ES, Sejd J, Palafox M, Monserrat L, Alemayehu S, Wu J, Sykes M, Aban A, Serra V and Nomanbhoy T. Direct CDKN2 modulation of CDK4 alters target engagement of CDK4 inhibitor drugs. Mol Cancer Ther 2019; 18: 771-79.
[27] Li Q, Jiang B, Guo J, Shao H, Del Priore IS, Chang Q, Kudo R, Li Z, Razavi P, Liu B, Boghossian AS, Rees MG, Ronan MM, Roth JA, Donovan KA, Palafox M, Reis-Filho JS, de Stanchina E, Fischer ES, Rosen N, Serra V, Koff A, Chodera JD, Gray NS and Chandarlapaty S. INK4 tumor suppressor proteins mediate resistance to CDK4/6 kinase inhibitors. Cancer Discov 2022; 12: 356-371.
[28] Lebok P, Roming M, Kluth M, Koop C, Özden C, Taskin B, Hussein K, Lebeau A, Witzel I, Wölber L, Geist S, Paluchowski P, Wilke C, Heilenkötter U, Müller V, Schmalfeldt B, Simon R, Sauter G, Terracciano L, Krech RH, von der Assen A and Burandt E. p16 overexpression and 9p21 deletion are linked to unfavorable tumor phenotype in breast cancer. Oncotarget 2016; 7: 81322-81331.
[29] Orlando S, Gallastegui E, Besson A, Abril G, Aligué R, Pujol MJ and Bachs O. p27Kip1 and p21Cip1 collaborate in the regulation of transcription by recruiting cyclin-Cdk complexes on the promoters of target genes. Nucleic Acids Res 2015; 43: 6860-73.
[30] Alvarez-Fernandez M and Malumbres M. Mechanisms of sensitivity and resistance to CDK4/6 inhibition. Cancer cell 2020; 37: 514-29.
[31] Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, Baselga J, Rosen N and Chandarlapaty S. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene 2017; 36: 2255-64.
[32] Tigan AS, Bellutti F, Kollmann K, Tebb G and Sexl V. CDK6-a review of the past and a glimpse into the future: from cell-cycle control to transcriptional regulation. Oncogene 2016; 35: 3083-91.
[33] Kollmann K, Heller G, Schneckenleithner C, Warsch W, Scheicher R, Ott RG, Schäfer M, Fajmann S, Schlederer M, Schiefer AI, Reichart U, Mayerhofer M, Hoeller C, Zöchbauer-Müller S, Kerjaschki D, Bock C, Kenner L, Hoefler G, Freissmuth M, Green AR, Moriggl R, Busslinger M, Malumbres M and Sexl V. A kinase-independent function of CDK6 links the cell cycle to tumor angiogenesis. Cancer Cell 2013; 24: 167-81.
[34] Wu X, Yang X, Xiong Y, Li R, Ito T, Ahmed TA, Karoulia Z, Adamopoulos C, Wang H, Wang L, Xie L, Liu J, Ueberheide B, Aaronson SA, Chen X, Buchanan SG, Sellers WR, Jin J and Poulikakos PI. Distinct CDK6 complexes determine tumor cell response to CDK4/6 inhibitors and degraders. Nat Cancer 2021; 2: 429-443.
[35] Iwata S, Tatsumi Y, Yonemoto T, Araki A, Itami M, Kamoda H, Tsukanishi T, Hagiwara Y, Kinoshita H, Ishii T, Nagase H and Ohira M. CDK4 overexpression is a predictive biomarker

for resistance to conventional chemotherapy in patients with osteosarcoma. Oncol Rep 2021; 46: 135.
[36] Olanich ME, Sun W, Hewitt SM, Abdullaev Z, Pack SD and Barr FG. CDK4 amplification reduces sensitivity to CDK4/6 inhibition in fusion-positive rhabdomyosarcoma. Clin Cancer Res 2015; 21: 4947-59.
[37] Augello MA, Burd CJ, Birbe R, McNair C, Ertel A, Magee MS, Frigo DE, Wilder-Romans K, Shilkrut M, Han S, Jernigan DL, Dean JL, Fatatis A, McDonnell DP, Visakorpi T, Feng FY and Knudsen KE. Convergence of oncogenic and hormone receptor pathways promotes metastatic phenotypes. J Clin Invest 2012; 123: 493-508.
[38] Ramos-García P, González-Moles MÁ, González-Ruiz L, Ruiz-Ávila I, Ayén A and Gil-Montoya JA. Prognostic and clinicopathological significance of cyclin D1 expression in oral squamous cell carcinoma: a systematic review and meta-analysis. Oral Oncol 2018; 83: 96-106.
[39] Capurso G, Festa S, Valente R, Piciucchi M, Panzuto F, Jensen RT and Delle Fave G. Molecular pathology and genetics of pancreatic endocrine tumours. J Mol Endocrinol 2012; 49: R37-R50.
[40] Sini MC, Doneddu V, Paliogiannis P, Casula M, Colombino M, Manca A, Botti G, Ascierto PA, Lissia A, Cossu A and Palmieri G. Genetic alterations in main candidate genes during melanoma progression. Oncotarget 2018; 9: 8531-8541.
[41] Khabaz MN, Abdelrahman AS, Butt NS, Al-Maghrabi B and Al-Maghrabi J. Cyclin D1 is significantly associated with stage of tumor and predicts poor survival in endometrial carcinoma patients. Ann Diagn Pathol 2017; 30: 47-51.
[42] Slattery ML, Herrick JS, Mullany LE, Samowitz WS, Sevens JR, Sakoda L and Wolff RK. The co-regulatory networks of tumor suppressor genes, oncogenes, and miRNAs in colorectal cancer. Genes Chromosomes Cancer 2017; 56: 769-787.
[43] Tchakarska G and Sola B. The double dealing of cyclin D1. Cell Cycle 2020; 19: 163-178.
[44] Benzeno S, Lu F, Guo M, Barbash O, Zhang F, Herman JG, Klein PS, Rustgi A and Diehl JA. Identification of mutations that disrupt phosphorylation-dependent nuclear export of cyclin D1. Oncogene 2006; 25: 6291-6303.
[45] Taylor-Harding B, Aspuria PJ, Agadjanian H, Cheon DJ, Mizuno T, Greenberg D, Allen JR, Spurka L, Funari V, Spiteri E, Wang Q, Orsulic S, Walsh C, Karlan BY and Wiedemeyer WR. Cyclin E1 and RTK/RAS signaling drive CDK inhibitor resistance via activation of E2F and ETS. Oncotarget 2015; 6: 696-714.
[46] Etemadmoghadam D, Au-Yeung G, Wall M, Mitchell C, Kansara M, Loehrer E, Batzios C, George J, Ftouni S, Weir BA, Carter S, Gresshoff I, Mileshkin L, Rischin D, Hahn WC, Waring PM, Getz G, Cullinane C, Campbell LJ and Bowtell DD. Resistance to CDK2 inhibitors is associated with selection of polyploid cells in CCNE1-amplified ovarian cancer. Clin Cancer Res 2013; 19: 5960-71.
[47] Konecny GE, Winterhoff B, Kolarova T, Qi J, Manivong K, Dering J, Yang G, Chalukya M, Wang HJ, Anderson L, Kalli KR, Finn RS, Ginther C, Jones S, Velculescu VE, Riehle D, Cliby WA, Randolph S, Koehler M, Hartmann LC and Slamon DJ. Expression of p16 and retinoblastoma determines response to CDK4/6 inhibition in ovarian cancer. Clin Cancer Res 2011; 17: 1591-602.
[48] Chaussepied M and Ginsberg D. Transcriptional regulation of AKT activation by E2F. Mol Cell 2004; 16: 831-7.
[49] Schachter MM, Merrick KA, Larochelle S, Hirschi A, Zhang C, Shokat KM, Rubin SM and Fisher RP. A Cdk7-Cdk4 T-loop phosphorylation cascade promotes G1 progression. Mol Cell 2013; 50: 250-60.
[50] Martin LA, Pancholi S and Ribas R. Resistance to palbociclib depends on multiple targetable mechanisms highlighting the potential of drug holidays and drug switching to improve therapeutic outcomes. Cancer Res 2017; 77: P3-03-09.
[51] Ghelli Luserna di Rorà A, Cerchione C, Martinelli G and Simonetti G. A WEE1 family business: regulation of mitosis, cancer progression, and therapeutic target. J Hematol Oncol 2020; 13: 126.
[52] Matheson CJ, Backos DS and Reigan P. Targeting WEE1 kinase in cancer. Trends Pharmacol Sci 2016; 37: 872-81.
[53] Fallah Y, Demas DM, Jin L, He W and Shajahan-Haq AN. Targeting WEE1 inhibits growth of breast cancer cells that are resistant to endocrine therapy and CDK4/6 inhibitors. Front Oncol 2021; 11: 681530.
[54] Liu JC, Granieri L, Shrestha M, Wang DY, Vorobieva I, Rubie EA, Jones R, Ju Y, Pellecchia G, Jiang Z, Palmerini CA, Ben-David Y, Egan SE, Woodgett JR, Bader GD, Datti A and Zacksenhaus E. Identification of CDC25 as a common therapeutic target for triple-negative breast cancer. Cell Rep 2018; 23: 112-126.
[55] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumors. Nature 2012; 490: 61-70.
[56] Efeyan A, Ortega-Molina A, Velasco-Miguel S, Herranz D, Vassilev LT and Serrano M. Induction of p53-dependent senescence by the MDM2 antagonist nutlin-3a in mouse cells of

fibroblast origin. Cancer Res 2007; 67: 7350-7.
[57] Lim E, Portman N and Alexandrou S. Therapeutic targeting of CDK4/6 inhibitor resistant breast cancer. Cancer Res 2018; 78: P4-04-12.
[58] Lee YR, Chen M and Pandolfi PP. The functions and regulation of the PTEN tumour suppressor: new modes and prospects. Nat Rev Mol Cell Biol 2018; 19: 547-562.
[59] Costa C, Wang Y, Ly A, Hosono Y, Murchie E, Walmsley CS, Huynh T, Healy C, Peterson R, Yanase S, Jakubik CT, Henderson LE, Damon LJ, Timonina D, Sanidas I, Pinto CJ, Mino-Kenudson M, Stone JR, Dyson NJ, Ellisen LW, Bardia A, Ebi H, Benes CH, Engelman JA and Juric D. PTEN loss mediates clinical cross-resistance to CDK4/6 and PI3Kalpha inhibitors in breast cancer. Cancer Discov 2020; 10: 72-85.
[60] Bencivenga D, Caldarelli I, Stampone E, Mancini FP, Balestrieri ML, Della Ragione F and Borriello A. p27(Kip1) and human cancers: a reappraisal of a still enigmatic protein. Cancer Lett 2017; 403: 354-65.
[61] Manivannan SN, Roovers J, Smal N, Myers CT, Turkdogan D, Roelens F, Kanca O, Chung HL, Scholz T, Hermann K, Bierhals T, Caglayan HS, Stamberger H; MAE Working Group of EuroEPINOMICS RES Consortium, Mefford H, de Jonghe P, Yamamoto S, Weckhuysen S and Bellen HJ. De novo FZR1 loss-of-function variants cause developmental and epileptic encephalopathies. Brain 2022; 145: 1684-1697.
[62] Ramanujan A and Tiwari S. APC/C and retinoblastoma interaction: cross-talk of retinoblastoma protein with the ubiquitin proteasome pathway. Biosci Rep 2016; 36: e00377.
[63] The I, Ruijtenberg S, Bouchet BP, Cristobal A, Prinsen MB, van Mourik T, Koreth J, Xu H, Heck AJ, Akhmanova A, Cuppen E, Boxem M, Muñoz J and van den Heuvel S. Rb and FZR1/Cdh1 determine CDK4/6-cyclin D requirement in C. elegans and human cancer cells. Nat Commun 2015; 6: 5906.
[64] Fujita T, Liu W, Doihara H and Wan Y. Regulation of Skp2-p27 axis by the Cdh1/anaphase-promoting complex pathway in colorectal tumorigenesis. Am J Pathol 2008; 173: 217-28.
[65] Peng Z, Gong Y and Liang X. Role of FAT1 in health and disease. Oncol Lett 2021; 21: 398.
[66] Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, Hsieh W, Sanchez-Vega F, Brown DN, Da Cruz Paula AF, Morris L, Selenica P, Eichenberger E, Shen R, Schultz N, Rosen N, Scaltriti M, Brogi E, Baselga J, Reis-Filho JS and Chandarlapaty S. Loss of the FAT1 tumor suppressor promotes resistance to CDK4/6 inhibitors via the Hippo pathway. Cancer Cell 2018; 34: 893-905.
[67] Mao P, Kusiel J and Cohen O. The role of FGF/FGFR axis in resistance to SERDs and CDK4/6 inhibitors in ER+ breast cancer. Cancer Res 2018; 78: PD4-01.
[68] Iida M, Nakamura M and Tokuda E. CDK6 might be a key factor for the efficacy of CDK4/6 inhibitor and the hormone sensitivity following acquired resistance. Cancer Res 2018; 78: 3541.
[69] Du B and Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules 2016; 21: 965.
[70] Vijayaraghavan S, Karakas C, Doostan I, Chen X, Bui T, Yi M, Raghavendra AS, Zhao Y, Bashour SI, Ibrahim NK, Karuturi M, Wang J, Winkler JD, Amaravadi RK, Hunt KK, Tripathy D and Keyomarsi K. CDK4/6 and autophagy inhibitors synergistically induce senescence in Rb positive cytoplasmic cyclin E negative cancers. Nat Commun 2017; 8: 15916.
[71] Smruthi V, Iman D and Jason PWC. Characterizing acquired resistance to palbociclib in breast cancer. Cancer Res 2017; 77: AM2017-60.
[72] Jain D and Aronow W. Cardiotoxicity of cancer chemotherapy in clinical practice. Hosp Pract (1995) 2019; 47: 6-15.
[73] Cao YN, Zheng LL, Wang D, Liang XX, Gao F and Zhou XL. Recent advances in microtubule-stabilizing agents. Eur J Med Chem 2018; 143: 806-28.
[74] He S, Roberts PJ, Sorrentino JA, Bisi JE, Storrie-White H, Tiessen RG, Makhuli KM, Wargin WA, Tadema H, van Hoogdalem EJ, Strum JC, Malik R and Sharpless NE. Transient CDK4/6 inhibition protects hematopoietic stem cells from chemotherapy-induced exhaustion. Sci Transl Med 2017; 9: eaal3986.
[75] Cretella D, Fumarola C, Bonelli M, Alfieri R, La Monica S, Digiacomo G, Cavazzoni A, Galetti M, Generali D and Petronini PG. Pre-treatment with the CDK4/6 inhibitor palbociclib improves the efficacy of paclitaxel in TNBC cells. Sci Rep 2019; 9: 13014.
[76] Zhang XH, Cheng Y, Shin JY, Kim JO, Oh JE and Kang JH. A CDK4/6 inhibitor enhances cytotoxicity of paclitaxel in lung adenocarcinoma cells harboring mutant KRAS as well as wild-type KRAS. Cancer Biol Ther 2013; 14: 597-605.
[77] Son KH, Kim MY, Shin JY, Kim J and Kang JH. Synergistic antitumor effect of taxanes and CDK4/6 inhibitor in lung cancer cells and mice harboring KRAS mutations. Anticancer Res 2021; 41: 4807-20.
[78] Robinson AM, Rathore R, Redlich NJ, Adkins DR, VanArsdale T, Van Tine BA and Michel LS. Cisplatin exposure causes c-Myc-dependent resistance to CDK4/6 inhibition in HPV-negative head and neck squamous cell carcinoma. Cell Death Dis 2019; 10: 867.

[79] Kim JY, Jayne LA, Bai Y, Feng MJHH, Clark MA, Chung S, W Christman J, Cianciolo RE and Pabla NS. Ribociclib mitigates cisplatin-associated kidney injury through retinoblastoma-1 dependent mechanisms. Biochem Pharmacol 2020; 177: 113939.
[80] Liu M, Cui L, Li X, Xia C, Li Y, Wang R, Ren F, Liu H and Chen J. PD-0332991 combined with cisplatin inhibits nonsmall cell lung cancer and reversal of cisplatin resistance. Thorac Cancer 2021; 12: 924-31.
[81] Hanker AB, Sudhan DR and Arteaga CL. Overcoming endocrine resistance in breast cancer. Cancer Cell 2020; 37: 496-513.
[82] Zhang H, Wang J, Li J, Zhou X, Yin L, Wang Y, Gu Y, Niu X, Yang Y, Ji H and Zhang Q. HMGB1 is a key factor for tamoxifen resistance and has the potential to predict the efficacy of CDK4/6 inhibitors in breast cancer. Cancer Sci 2021; 112: 1603-13.
[83] Finn RS, Dieras V and Rugo HS. Palbociclib (PAL) + letrozole (L) as first-line (1L) therapy (tx) in estrogen receptor-positive (ER+)/human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (ABC): efficacy and safety across patient (pt) subgroups. J Clin Oncol 2017; 35: 1039.
[84] Schiavon G, Hrebien S, Garcia-Murillas I, Cutts RJ, Pearson A, Tarazona N, Fenwick K, Kozarewa I, Lopez-Knowles E, Ribas R, Nerurkar A, Osin P, Chandarlapaty S, Martin LA, Dowsett M, Smith IE and Turner NC. Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer. Sci Transl Med 2015; 7: 313ra182.
[85] Dustin D, Gu G and Fuqua SAW. ESR1 mutations in breast cancer. Cancer 2019; 125: 3714-28.
[86] Turner NC, Slamon DJ, Ro J, Bondarenko I, Im SA, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, Iwata H, Harbeck N, Loibl S, André F, Puyana Theall K, Huang X, Giorgetti C, Huang Bartlett C and Cristofanilli M. Overall survival with palbociclib and fulvestrant in advanced breast cancer. N Engl J Med 2018; 379: 1926-36.
[87] Bedard PL, Hyman DM, Davids MS and Siu LL. Small molecules, big impact: 20 years of targeted therapy in oncology. Lancet 2020; 395: 1078-88.
[88] Zafar A, Wang W, Liu G, Wang X, Xian W, McKeon F, Foster J, Zhou J and Zhang R. Molecular targeting therapies for neuroblastoma: progress and challenges. Med Res Rev 2021; 41: 961-1021.
[89] Curtin NJ and Szabo C. Poly(ADP-ribose) polymerase inhibition: past, present and future. Nat Rev Drug Discov 2020; 19: 711-36.
[90] Wu C, Peng S, Pilié PG, Geng C, Park S, Manyam GC, Lu Y, Yang G, Tang Z, Kondraganti S, Wang D, Hudgens CW, Ledesma DA, Marques-Piubelli ML, Torres-Cabala CA, Curry JL, Troncoso P, Corn PG, Broom BM and Thompsonl TC. PARP and CDK4/6 inhibitor combination therapy induces apoptosis and suppresses neuroendocrine differentiation in prostate cancer. Mol Cancer Ther 2021; 20: 1680-91.
[91] Tempka D, Tokarz P, Chmielewska K, Kluska M, Pietrzak J, Rygielska Z, Virág L and Robaszkiewicz A. Downregulation of PARP1 transcription by CDK4/6 inhibitors sensitizes human lung cancer cells to anticancer drug-induced death by impairing OGG1-dependent base excision repair. Redox Biol 2018; 15: 316-26.
[92] Zhu X, Chen L, Huang B, Li X, Yang L, Hu X, Jiang Y, Shao Z and Wang Z. Efficacy and mechanism of the combination of PARP and CDK4/6 inhibitors in the treatment of triple-negative breast cancer. J Exp Clin Cancer Res 2021; 40: 122.
[93] Abdeldayem A, Raouf YS, Constantinescu SN, Moriggl R and Gunning PT. Advances in covalent kinase inhibitors. Chem Soc Rev 2020; 49: 2617-87.
[94] La Monica S, Fumarola C, Cretella D, Bonelli M, Minari R, Cavazzoni A, Digiacomo G, Galetti M, Volta F, Mancini M, Petronini PG, Tiseo M and Alfieri R. Efficacy of the CDK4/6 dual inhibitor abemaciclib in EGFR-mutated NSCLC Cell lines with different resistance mechanisms to osimertinib. Cancers (Basel) 2020; 13: 6.
[95] Qin Q, Li X, Liang X, Zeng L, Wang J, Sun L and Zhong D. CDK4/6 inhibitor palbociclib overcomes acquired resistance to third-generation EGFR inhibitor osimertinib in non-small cell lung cancer (NSCLC). Thorac Cancer 2020; 11: 2389-97.
[96] Chen Z, Xu Y, Gong J, Kou F, Zhang M, Tian T, Zhang X, Zhang C, Li J, Li Z, Lai Y, Zou J, Zhu X, Gao J and Shen L. Pyrotinib combined with CDK4/6 inhibitor in HER2-positive metastatic gastric cancer: a promising strategy from AVATAR mouse to patients. Clin Transl Med 2020; 10: e148.
[97] Wang Y, Yuan X, Li J, Liu Z, Li X, Wang Z, Wei L, Li Y and Wang X. The synergistic effects of SHR6390 combined with pyrotinib on HER2+/HR+ breast cancer. Front Cell Dev Biol 2021; 9: 785796.
[98] Agostinetto E, Debien V, Marta GN, Lambertini M, Piccart-Gebhart M and de Azambuja E. CDK4/6 and PI3K inhibitors: a new promise for patients with HER2-positive breast cancer. Eur J Clin Invest 2021; 51: e13535.
[99] Alves CL, Ehmsen S, Terp MG, Portman N, Tuttolomondo M, Gammelgaard OL, Hundebøl MF, Kaminska K, Johansen LE, Bak M, Honeth G,

Bosch A, Lim E and Ditzel HJ. Co-targeting CDK4/6 and AKT with endocrine therapy prevents progression in CDK4/6 inhibitor and endocrine therapy-resistant breast cancer. Nat Commun 2021; 12: 5112.
[100] Dang F, Nie L, Zhou J, Shimizu K, Chu C, Wu Z, Fassl A, Ke S, Wang Y, Zhang J, Zhang T, Tu Z, Inuzuka H, Sicinski P, Bass AJ and Wei WY. Inhibition of CK1ε potentiates the therapeutic efficacy of CDK4/6 inhibitor in breast cancer. Nat Commun 2021; 12: 5386.
[101] Babar Q, Saeed A, Tabish TA, Pricl S, Townley H and Thorat N. Novel epigenetic therapeutic strategies and targets in cancer. Biochim Biophys Acta Mol Basis Dis 2022; 1868: 166552.
[102] Kulis M and Esteller M. DNA methylation and cancer. Adv Genet 2010; 70: 27-56.
[103] Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nat Rev Genet 2018; 19: 81-92.
[104] Li P, Zhang X, Gu L, Zhou J and Deng D. P16 methylation increases the sensitivity of cancer cells to the CDK4/6 inhibitor palbociclib. PLoS One 2019; 14: e0223084.
[105] Duan R, Du W and Guo W. EZH2: a novel target for cancer treatment. J Hematol Oncol 2020; 13: 104.
[106] Müller A, Dickmanns A, Resch C, Schäkel K, Hailfinger S, Dobbelstein M, Schulze-Osthoff K and Kramer D. The CDK4/6-EZH2 pathway is a potential therapeutic target for psoriasis. J Clin Invest 2020; 130: 5765-81.
[107] Li Y, Ren Y, Wang Y, Tan Y, Wang Q, Cai J, Zhou J, Yang C, Zhao K, Yi K, Jin W, Wang L, Liu M, Yang J, Li M and Kang C. A Compound AC-1Q3QWB selectively disrupts HOTAIR-mediated recruitment of PRC2 and enhances cancer therapy of DZNep. Theranostics 2019; 9: 4608-23.
[108] Shi J, Lv S, Wu M, Wang X, Deng Y, Li Y, Li K, Zhao H, Zhu X and Ye M. HOTAIR-EZH2 inhibitor AC1Q3QWB upregulates CWF19L1 and enhances cell cycle inhibition of CDK4/6 inhibitor palbociclib in glioma. Clin Transl Med 2020; 10: 182-98.
[109] Jarrold J and Davies CC. PRMTs and arginine methylation: cancer's best-kept secret? Trends Mol Med 2019; 25: 993-1009.
[110] Hwang JW, Cho Y, Bae GU, Kim SN and Kim YK. Protein arginine methyltransferases: promising targets for cancer therapy. Exp Mol Med 2021; 53: 788-808.
[111] Ramaiah MJ, Tangutur AD and Manyam RR. Epigenetic modulation and understanding of HDAC inhibitors in cancer therapy. Life Sci 2021; 277: 119504.
[112] Zupkovitz G, Grausenburger R, Brunmeir R, Senese S, Tischler J, Jurkin J, Rembold M, Meunier D, Egger G, Lagger S, Chiocca S, Propst F, Weitzer G and Seiser C. 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.
[113] Lee J, Lim B and Pearson T. 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.
[114] Liu Z, Wang P, Chen H, Wold EA, Tian B, Brasier AR and Zhou J. Drug discovery targeting bromodomain-containing protein 4. J Med Chem 2017; 60: 4533-58.
[115] Ge JY, Shu S, Kwon M, Jovanović B, Murphy K, Gulvady A, Fassl A, Trinh A, Kuang Y, Heavey GA, Luoma A, Paweletz C, Thorner AR, Wucherpfennig KW, Qi J, Brown M, Sicinski P, McDonald TO, Pellman D, Michor F and Polyak K. Acquired resistance to combined BET and CDK4/6 inhibition in triple-negative breast cancer. Nat Commun 2020; 11: 2350.
[116] Margueron R, Li G, Sarma K, Blais A, Zavadil J, Woodcock CL, Dynlacht BD and Reinberg D. Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. Mol Cell 2008; 32: 503-18.
[117] D'Oto A, Fang J, Jin H, Xu B, Singh S, Mullasseril A, Jones V, Abu-Zaid A, von Buttlar X, Cooke B, Hu D, Shohet J, Murphy AJ, Davidoff AM and Yang J. KDM6B promotes activation of the oncogenic CDK4/6-pRB-E2F pathway by maintaining enhancer activity in MYCN-amplified neuroblastoma. Nat Commun 2021; 12: 7204.
[118] Li LX, Zhou JX, Wang X, Zhang H, Harris PC, Calvet JP and Li X. Cross-talk between CDK4/6 and SMYD2 regulates gene transcription, tubulin methylation, and ciliogenesis. Sci Adv 2020; 6: eabb3154.
[119] Fassl A, Brain C, Abu-Remaileh M, Stukan I, Butter D, Stepien P, Feit AS, Bergholz J, Michowski W, Otto T, Sheng Q, Loo A, Michael W, Tiedt R, DeAngelis C, Schiff R, Jiang B, Jovanovic B, Nowak K, Ericsson M, Cameron M, Gray N, Dillon D, Zhao JJ, Sabatini DM, Jeselsohn R, Brown M, Polyak K and Sicinski P. Increased lysosomal biomass is responsible for the resistance of triple-negative breast cancers to CDK4/6 inhibition. Sci Adv 2020; 6: eabb2210.
[120] Békés M, Langley DR and Crews CM. PROTAC targeted protein degraders: the past is prologue. Nat Rev Drug Discov 2022; 21: 181-200.
[121] Caksa S and Aplin AE. PROactively TACkling CDK4/6 therapy resistance. Nat Cancer 2021; 2: 372-3.

[122] Schreiber RD, Old LJ and Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011; 331: 1565-70.
[123] Waldman AD, Fritz JM and Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol 2020; 20: 651-68.
[124] Teh JLF and Aplin AE. Arrested developments: CDK4/6 inhibitor resistance and alterations in the tumor immune microenvironment. Clin Cancer Res 2019; 25: 921-7.
[125] Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, Chhabra S, Huang W, Liu H, Aref AR, Ivanova E, Paweletz CP, Bowden M, Zhou CW, Herter-Sprie GS, Sorrentino JA, Bisi JE, Lizotte PH, Merlino AA, Quinn MM, Bufe LE, Yang A, Zhang Y, Zhang H, Gao P, Chen T, Cavanaugh ME, Rode AJ, Haines E, Roberts PJ, Strum JC, Richards WG, Lorch JH, Parangi S, Gunda V, Boland GM, Bueno R, Palakurthi S, Freeman GJ, Ritz J, Haining WN, Sharpless NE, Arthanari H, Shapiro GI, Barbie DA, Gray NS and Wong KK. CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. Cancer Discov 2018; 8: 216-33.
[126] Lelliott EJ, Kong IY, Zethoven M, Ramsbottom KM, Martelotto LG, Meyran D, Zhu JJ, Costacurta M, Kirby L, Sandow JJ, Lim L, Dominguez PM, Todorovski I, Haynes NM, Beavis PA, Neeson PJ, Hawkins ED, McArthur GA, Parish IA, Johnstone RW, Oliaro J, Sheppard KE, Kearney CJ and Vervoort SJ. CDK4/6 inhibition promotes antitumor immunity through the induction of T-cell memory. Cancer Discov 2021; 11: 2582-601.
[127] Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, Khan N, Ubellacker JM, Xie S, Metzger-Filho O, Hoog J, Ellis MJ, Ma CX, Ramm S, Krop IE, Winer EP, Roberts TM, Kim HJ, McAllister SS and Zhao JJ. CDK4/6 inhibition triggers anti-tumour immunity. Nature 2017; 548: 471-5.