Targeting Cyclin Dependent Kinase 4/6 for Cancer Therapy

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Abstract: One fundamental hallmark of cancer is uncontrolled cell proliferation. In order to inhibit tumor growth, understanding the mechanisms of disease pathogenesis and the role of cell-cycle regulators which control the division process of cancer cells is necessary, specially CDK4/6, which have been approved as effective therapeutic targets in clinical studies. To date, five CDK4/6 inhibitors have been approved by the U.S. Food and Drug Administration (FDA) and National Medical Products Administration (NMPA). As expected, CDK4/6 inhibitors arrest tumor cells in the G1 phase of the cell cycle. Although undesired, mechanisms of acquired resistance to CDK4/6 inhibitors are beginning to emerge, and extending the use of CDK4/6 inhibitors beyond HR-positive breast cancer is still under consideration and practice. In this assay, we highlight the biological functions of CDK4/6 in the control of cell cycle progression in normal cells and summarize the multiple mechanisms by which the dysregulation of the CDK4/6 pathway of cancer cells. This review also provides a general overview of CDK4/6 inhibitors clinically approved. Instead of monotherapies, combination therapies with CDK inhibitors may especially provide promising results for cancer therapy. We also demonstrate the possible combination with available targeted therapies, immunotherapy, or classical chemotherapy to improve future therapeutic uses of CDK4/6 inhibition in a variety of cancers in clinical trials.

1. Introduction

Cancer is one of the leading diseases of mortality worldwide accounting for nearly 10 million deaths in 2020. Traditional cancer treatment methods (i.e. surgery, radiation, and chemotherapy) have been proven to indiscriminately destroy both normal and malignant cells, resulting in significant toxicities and side effects[1]. The sustained cell proliferation caused by uncontrolled cell division is one of the key pathological manifestations of cancer transformation. Therefore, inhibiting abnormal cell division and proliferation is a promising strategy in cancer treatment. In particular, cyclin-dependent kinases (CDKs) are critical in the regulation of cell division and proliferation. Leland S. Hartwell, Paul M. Nurse, and R Timothy Hunt had been acknowledged with a Nobel prize in Physiology or Medicine for their discovery of “key regulators of the cell cycle” in 2001. Due to
CDK’s key function in regulating cell division and proliferation, many drugs targeting CDK have been developed over the past 20 years. Despite promising preclinical results, the first and second generation CDK inhibitors were discontinued as these nonselective pan-CDK inhibitors led to serious cytotoxic effects on normal cells. However, the third-generation of CDK inhibitors which exhibit selectivity for CDK4/6 over other CDKs (Table 1), have received regulatory approval from FDA and NMPA for the treatment of patients with HR+ breast cancer or prevention of myelosuppression due to platinum/etoposide or topotecan chemotherapy in adults with spread-stage small cell lung cancer. In this assay, we discuss the important roles of CDK4/6 in the regulation of cell cycle progression in normal cells and summarize the multiple mechanisms by which the dysregulation of the CDK4/6 pathway results in the uncontrolled proliferation of cancer cells. In particular, we discuss the rationale for selectively inhibiting CDK4/6 for cancer treatment and review the recent advances in the development of highly selective CDK4/6 inhibition in clinical trials.

2. The biology of CDK4/6 pathways

Cyclin-dependent kinases (CDKs) are serine/threonine protein kinases, which function with their co-partners (cyclins) to promote cell progression during cell division. At present, more than 20 different CDKs have been found which is involved in many physiological processes. According to their different functions, CDKs are mainly divided into two categories, one is involved in cell cycle regulation, mainly including CDK1/2/4/6 and the other plays individual roles in critical cellular processes via regulation of gene transcription, mainly including CDK7/8/9/10/11.

The cell cycle is universally divided into four phases: G1, S, G2, and M. The components of CDK4/6 pathways play a critical role in cell cycle control from G0/G1 to the S phase (Figure 1). CDK4 shares 71% amino acid homology with CDK6 and can bind to all three isoforms of cyclin D (cyclin D1, D2, D3) that facilitate the progression of cells through the early G1 phase of the cell cycle. When the mitogenic signals stimulate D-type cyclins level increase, the D-type cyclin can form an active complex with CDK4/6, which subsequently hyper-phosphorylates retinoblastoma-associated protein (Rb), and the Rb-E2F transcription inhibitor complex is depolymerized to release the E2F transcription factor. The free E2F then activates the related genes needed for DNA replication and drives the cell to enter the S phase. In addition, CDK4/6 activation and inhibition are regulated by some CDK inhibitors (CKIs). CKIs are composed of two distinct classes of regulatory subunits, the Cip/Kip family, comprising p21Cip1 (CDKN1A), p27Kip1 (CDKN1B), and p57Kip2 (CDKN1C), and the INK4 family, including p15INK4b (CDKN2B), p16INK4a (CDKN2A), p18INK4c (CDKN2C) and p19INK4d (CDKN2D). Cip/Kip family members can act on a broader spectrum of CDK-cyclin complexes and have both a positive and negative regulatory function depending on the complex proteins and phosphorylation status.

Figure 1: Cell progression through G0/G1-S phase regulated by CDK4/6-RB pathways
When the mitogenic signals stimulate CDK4/6 and D-type cyclins form active complex with CDK4/6, which subsequently phosphorylating RB leading to RB-E2F complex partially relief suppression of E2Fs to allow cascades of genes expression including cyclin E, which activates another kinase CDK2- leading to hyperphosphorylation of RB fully releasing suppression of E2Fs and DNA replication allowing cells to exit G1 to enter S phase. (The pictures are drawn with science slides suite

3. The pathology of CDK 4/6 pathway in cancer

The cancer genome atlas program (TCGA) data shows that CDK4/6 pathway deregulation is widely spread in 31 kinds of cancers. CDK4/6 and D-type cyclin amplification have oncogenic potential. Besides, loss of the endogenous CDK4/6 inhibitors or RB-family are also observed in some tumors. Comprehensive genomic analyses show that abnormalities of CDK4/6-RB pathways are associated with multiple tumor occurrences suggesting that CDK4/6 might be suitable targets for therapeutic intervention.

3.1 CDK4

CDK4 belongs to a member of the Ser/Thr protein kinase family and functions as a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression. CDK4 amplification is present in ~4% of cancer cases worldwide [2]. CDK4 amplification is a predictive biomarker for use of palbociclib and ribociclib in CDK4-amplified liposarcoma and neuroblastoma patients. While in rhabdomyosarcoma, CDK4 overexpression may reduce the sensitivity of ribociclib treatment. Besides, High CDK4 T172 phosphorylation in breast cancer was more sensitive to palbociclib treatment [3]. Additionally, CDK4 R24C point mutation in melanomas leads to CDK4 being insensitive to inhibition of INK4 family members [4]. Besides, High CDK4 expression in mouse epidermis can lead to epidermal hyperplasia, hypertrophy, and severe dermal fibrosis which demonstrates that CDK4 has higher oncogenic activity, revealing a potential use of CDK4 as a therapeutic target [5].

3.2 CDK6

CDK6 was discovered several years after CDK4. For a long time, it had been regarded as a mere homolog of CDK4 with redundant functions in the initiation of the cell cycle. In fact, CDK6 functions both as a cell-cycle kinase and as a transcriptional regulator. The effects of CDK6 on tumor growth are difficult to predict, as CDK6 both stimulates the transcription of tumor suppressors such as p16\(^{INK4a}\) and increases the transcription of factors that enhance tumor formation and proliferation. Recent studies have revealed that CDK6 is expressed at high levels and is systematically correlated with poor prognosis in many types of tumors. For example, CDK6 upregulation is revealed to be positively correlated with the stage and invasive behavior of bladder cancer[6].CDK6 overexpression on chromosome 7q21.2 is also associated with an adverse prognosis in medulloblastoma [7] and myxofibrosarcoma [8]. Besides, overexpression of CDK6 has been reported in T-cell lymphoblastic lymphoma, and leukemia and B-lymphoid malignancies. An inverse correlation of CDK6 and p16\(^{INK4A}\) has been detected in most human lymphoid malignancies. These data indicated that CDK6 may act as an oncogene and play a critical role in tumor development and progression. However, other findings imply that CDK6 upregulation is a targetable resistance mechanism for lenalidomide in multiple myeloma [9] and abemaciclib in breast cancer [10] which may hamper its clinical application.
3.3 Cyclin D

D-type cyclins (cyclins D1-D3) are essential for cell cycle progression at G1-S, where they bind to and activate cyclin-dependent kinases (CDK4 and CDK6) to trigger phosphorylation of the retinoblastoma protein and initiation of DNA synthesis and show substantial amino acid sequence similarity, and are expressed in an overlapping, redundant fashion in all proliferating cell types [11]. A comprehensive analysis of many human cancer types revealed that the gene encoding cyclin D1 represents the second most frequently amplified locus in the human cancer genome [12]. Cyclin D1 has been regarded as a proto-oncogene associated with the uncontrolled proliferation of tumor cells. For example, mice lacking cyclin D1 are resistant to ErbB2-driven mammary adenocarcinomas, while cyclin D3-null animals are refractory to Notch1-driven T-ALL [13]. For patients in diffuse large B-cell lymphoma, the higher cyclin D3 expression was associated with higher tumor grades and shorter overall survival [14]. Moreover, up to 95% of melanoma tumor cells also express cyclin D3, whose downregulation predicts poor clinical outcomes for the superficial subtypes [15], and can be used as a biomarker for the identification of patients most likely to respond. Besides, the cyclin D1 3’UTR mutation in 5% of endometrial cancer has been shown to increase cyclin D1 expression and can activate D-type cyclins, which also enhances the sensitivity to the CDK4/6 inhibitor abemaciclib [16]. However, SMARCA4, encoding an SWI/SNF catalytic ATPase subunit, inactivated by mutations in non-small cell lung cancer (NSCLC) and ovarian cancer causes cyclin D1 deficiency leading to high sensitivity to CDK4/6 inhibitors [17].

3.4 RB1

RB1 is the first tumor suppressor gene identified, which functions to repress many transcriptional genes that are required for progression through S-phase, mitosis, and cytokinesis. Retinoblastoma (Rb) is a negative regulator of the cell cycle by binding to E2F transcription factors and preventing cell division in this way. Thus, the Rb protein is a major G1 checkpoint, blocking S-phase entry and cell growth. However, more than 30% of tumors have gene mutations in the RB1 pathway, and single copy loss on chromosome 13q encompassing the RB1 locus is popular in many cancers. Besides, other somatic RB1 mutations including substitution RB1 exon 8, exon 22 substitution, exon 19 deletion, exon 3 insertion, and RB1 exon 16 H483Y mutation after exposure to palbociclib or ribociclib, in patients with hormone receptor-positive (HR+) breast cancer confers these drugs therapeutic resistance [18]. By analyzing circulating tumor DNA (ctDNA) from breast cancer patients who had been treated with a CDK4/6 inhibitor for several months, Condorelli et al. detected acquired RB1 mutations, and these alterations could lead to Rb functional loss, conferring CDK4/6 inhibitor resistance [18]. However, in advanced bladder cancer, regardless of Rb status, palbociclib as a monotherapy or in combination with cisplatin has demonstrated significant efficacy and antitumor effects by inhibiting FOXM1 phosphorylation [19]. However, it seems like RB1 may not need to be present in all cases, as CDK4/6 inhibitors were still effective in RB1-deficient human liver cancer cell lines and a mouse model of liver cancer with genetic deletion of RB1 [20]. In this case, p107 protein stability was dramatically increased upon CDK4/6 inhibition, indicating that other pocket proteins can compensate for the loss of RB in mediating cell cycle arrest.

3.5 E2F

The CDK-RB1-E2F axis is the fundamental signaling pathway that controls cell cycle progression. The E2F gene family is the main axis effector. The E2F family of transcription factors is composed of eight genes that are crucial for coordinating the cell cycle. E2Fs bind the members of the pRB family with different specificities: E2F1, E2F2, and E2F3 bind exclusively to pRB, E2F5...
preferentially binds p130, and E2F4 is unique in its capacity to bind all the pocket proteins. When mitogenic signals trigger proliferation, a complex composed of cyclins and cyclin-dependent kinases forms, and then phosphorylates and inactivates pocket proteins, removing repressive complexes from E2F and E2F target promoters which induces the transcription of G1-S phase cell cycle genes, resulting in cell cycle progression. Except for cell cycle control and regulation, the E2F family also play important role in maintaining chromosome stability and gene replicate, DNA damage response and apoptosis, angiogenesis, extracellular matrix remodeling, and tumor invasion and metastasis.

Almost all malignancies can cause increased oncogenic E2F activity, which leads to uncontrolled cell growth and proliferation even in the context of MYC induction or functional inactivation of RB. In letrozole-resistant ER+ breast cancers, the activity of E2F4 was increased, and most of the E2F4 target genes were upregulated; in addition, treatment with palbociclib in letrozole-resistance patients before surgery significantly decreased the expression of E2F4 target genes [21]. It seems like inhibiting E2Fs activities is useful for cancer treatment. Two small molecular inhibitors targeting E2F4-TFDP2 complex HLM006474 and ly101-4B can downregulate E2F target expression and has anti-proliferative and pro-apoptotic activity in multiple cancer cell lines and reduces tumor growth in many cancer models[22, 23]. However, the overexpression of E2F activating transcription factors can bypass CDK4/6 inhibition by driving DNA replication and mitosis, which is involved in the inherent resistance to CDK4/6 inhibitors. Besides, in BRAF-mutant and NRAS-mutant melanomas, E2F reactivation has been identified as the mechanism by which tumors acquire resistance to combined MEK inhibitors and CDK4/6 inhibitors [24].

4. CDK 4/6 inhibitors for cancer therapy

Malignant tumors usually have the following characteristics: inactivation of apoptotic programs, formation of new blood vessels, evasion of immune surveillance, amplification of oncogenes, abnormal adhesion function, and cell immortalization. Among them, the targeting regulation of tumor cell cycle to change the apoptosis state of tumor cells has been clinically proven effective, and the specific representative variety is the CDK family inhibitor, especially CDK4/6 inhibitors. CDK4/6 inhibitors can block the process of cells from G1 phase to S phase, reduce tumor cell proliferation and inhibit abnormal cell replication. Until now, five CDK4/6 inhibitors have been approved. Each agent has demonstrated its efficacy, but differences among the five drugs exist, particularly in their adverse-event profiles and candidate (table 1). Here we review the highly specific, potent ATP-competitive CDK4/6 inhibitors which have been successful in clinical studies.

4.1 Palbociclib

The discovery of palbociclib is a big step in the design of specific CDK inhibitors. Palbociclib is an ATP-binding competitor which is highly selective for CDK4/6 over other 36 protein kinases and can induce G1 arrest with reduction of phospho-Ser 780/795 on the Rb protein and downregulation of genes driven by the E2Fs [25]. It can also inhibit epithelia-mesenchymal transition (EMT) and metastasis via the c-Jun/COX-2 signaling pathway [26]. It has shown prominent effects in many clinical and preclinical tumors.

Palbociclib is an orally bioavailable drug that showed good pharmacokinetic properties in many patients with retinoblastoma protein (Rb)-positive advanced solid tumors. In this phase 2 (PALOMA-1) study, which compared palbociclib plus letrozole with letrozole alone as initial therapy for ET-naive advanced breast cancer patients the palbociclib plus letrozole group show significantly improved progression-free survival (PFS) versus letrozole alone (20.2 months vs. 10.2 months, respectively; HR=0.488, 95% CI 0.319-0.748; one-sided p=0.0004) [27]. In 2015, FDA granted accelerated approval for palbociclib in combination with letrozole as a frontline treatment for
postmenopausal women with ER-positive/HER2-negative metastatic breast cancer in the USA. Palbociclib is the first CDK4/6 inhibitor to achieve regulatory approval.

These results led to two phase 2 trials: PALOMA-2 and PALOMA-3 further evaluating palbociclib’s effect in this patient population. PALOMA-2 is an international, randomized, double-blind, placebo-controlled, clinical trial that randomized 666 postmenopausal women (2:1) to palbociclib plus letrozole or placebo plus letrozole. Palbociclib 125 mg or placebo was administered orally once daily for 21 consecutive days, followed by 7 days off. Letrozole 2.5 mg was administered orally once daily. Treatment continued until disease progression or unacceptable toxicity. Initial results of PALOMA-2 confirmed the effects of palbociclib plus letrozole, demonstrating similar improved progression-free survival (PFS) versus letrozole alone (24.8 months vs. 14.5 months, respectively; HR=0.58; 95% CI, 0.46-0.72; p<0.001) comparable to PALOMA-1 study. A long-term follow-up study at 38 months further confirmed palbociclib’s effect on PFS, with an increase to 27.6 months versus 14.5 months (HR=0.563; 95% CI, 0.461-0.687; p<0.0001)[28, 29].

Based on the data of PALOMA-2 study, on March 31, 2017, the U.S. Food and Drug Administration granted regular approval to palbociclib for the treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer in combination with an aromatase inhibitor as initial endocrine-based therapy in postmenopausal women. Shortly after the start of PALOMA-2, another phase 2 trial, PALOMA-3, was begun to assess the safety and efficacy of palbociclib plus fulvestrant in premenopausal or postmenopausal women with HR+ advanced breast cancer that progressed on prior ET. The initial report after 5.6 months of follow-up showed an improvement in median PFS (9.2 months vs. 3.8 months), with a 58% reduction in progression (HR=0.42; 95% CI, 0.32-0.56; P <0.001). A prespecified analysis of OS in the PALOMA-3 trial, using a median follow-up of 44.8 months, found that the differences in OS for the entire intent-to-treat group did not reach statistical significance, but the combination did prolong OS by 10 months in patients who were sensitive to previous ET [30, 31]. Data from the phase 2 clinical trial, PALOMA-3, on which this National Institute for Health and Care Excellence (NICE) recommendation is based, Pfizer has announced that palbociclib has been recommended by the NICE for use in combination with fulvestrant for the treatment of women with hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) locally advanced or metastatic breast cancer who have received prior endocrine therapy in 2019.

### 4.2 Ribociclib

Ribociclib is another orally available, selective CDK4/6 inhibitor which can also induce dephosphorylation of Rb and G1 cell cycle arrest. Mar 13th in 2017, FDA has approved that ribociclib in combination with aromatase inhibitors can be used as first-line treatment for advanced HR+/HER2- metastatic breast cancer for postmenopausal women. Ribociclib first received FDA approval after the MONALEESA-2 trial which found that PFS was improved when the drug was given in combination with letrozole versus letrozole alone. Rates of locally assessed PFS versus letrozole alone after 18 months were 63.0% (95% CI, 54.6-70.3) and 42.2% (95% CI, 34.8-49.5), respectively [32]. Ribociclib is also being investigated (in MONALEESA-3) in combination with fulvestrant in treatment-naïve patients and those who relapsed after ET. The initial report revealed improved PFS for the combination compared with fulvestrant alone (20.5 months vs. 12.8 months, respectively; HR=0.593; 95% CI, 0.480-0.732; p <0.001), which resulted in FDA approval of this combination in 2018 [33]. This clinical study also proved the OS benefit of ribociclib plus fulvestrant compared with fulvestrant alone, with an estimated OS at 42 months of 57.8% versus 45.9%, respectively, and a 28% reduction in relative risk of death (HR=0.72; 95% CI, 0.57-0.92; p =0 .00455)[34]. Recently, Novartis announced the MONALEESA-3 study updated median overall survival (OS) results from a phase 2
study in women with advanced breast cancer. Results of an exploratory analysis of OS (overall survival) after an additional 16.9 months of follow-up showed that after a median follow-up of 56.3 months, the median OS was 53.7 months in the ribociclib plus fulvestrant group and 41.5 months in the fulvestrant monotherapy group (HR=0.73; 95% CI: 0.59-0.90). In terms of time to first chemotherapy, the time to first chemotherapy was 48.1 months and 28.8 months in the ribociclib plus fulvestrant group compared with the placebo plus fulvestrant group, respectively (HR=0.70; 95% CI: 0.57-0.88) [35].

Moreover, a third clinical trial, MONALEESA-7, is currently the only phase 2 trial to evaluate the efficacy and safety of a CDK4/6 inhibitor as first-line therapy for pre- or perimenopausal women who have metastatic breast cancer. In this trial, ribociclib plus tamoxifen or a nonsteroidal AI (NSAI)-such as letrozole or anastrozole plus goserelin significantly improved PFS by 23.8 months versus 13 months for the placebo group (HZR, 0.55; 95% CI, 0.44-0.69; P <.0001), and the combination led to the expanded indication that includes pre-and perimenopausal women[36].

4.3 Abemaciclib

The third CDK4/6 inhibitor FDA approved is abemaciclib. Abemaciclib is another orally bioavailable drug that selectively inhibited CDK4/6 in the nanomolar range. Abemaciclib can also inhibit phosphorylation of RB inducing G1 arrest and suppressing the expression of several Rb-E2F-regulated genes. This agent is indicated in combination with an AI as initial ET in postmenopausal women with advanced breast cancer, or in combination with fulvestrant in pre-, peri-, or postmenopausal women with disease progression following ET. Abemaciclib is also approved as monotherapy in men and women with disease progression following ET and prior chemotherapy in the metastatic setting.

The agent initially gained approval based on results from MONARCH-1 demonstrating promising clinical activity after 12 months, with an overall response rate (ORR) of 19.7% (95% CI, 13.3-27.5) and a median PFS of 6 months[37]. During this time, abemaciclib was also being investigated in MONARCH-2 for pre-, peri-, and postmenopausal women who progressed on ET. This study demonstrated statistically significant PFS benefit with a combination of abemaciclib plus fulvestrant versus fulvestrant alone (16.4 months vs. 9.3 months; HR=0.553; 95% CI, 0.449-0.681; p<0.001)[38]. An extended follow-up study with a median duration of 47.7 months reported slightly improved PFS results and OS benefit with the abemaciclib combination versus placebo versus fulvestrant (46.7 months vs. 37.3 months; 95% CI, 0.606-0.945; p=0.01)[39]. Shortly after the initiation of the two abemaciclib trials, MONARCH-3 was started. This trial found a statistically significant improvement in PFS and ORR with abemaciclib plus an NSAI compared with the placebo-plus-NSAI arm as first-line treatment for postmenopausal women. An updated report with 8.9 months of additional follow-up further confirmed the PFS and ORR results of the initial analysis [40, 41].

Overall, abemaciclib has been noted to be the most potent CDK4/6 inhibitor, with excellent central nervous system activity due to its structure, allowing it to cross the blood-brain barrier and remain on target longer. This may be beneficial for patients with brain metastases. Besides, it has also been shown to effectively cross the blood-brain barrier (BBB) in preclinical human brain models. Brain exposure of two selective dual CDK4 and CDK6 inhibitors and the antitumor activity of CDK4 and CDK6 inhibition in combination with temozolomide in an intracranial glioblastoma xerograph shows that abemaciclib had brain area under the curve (0-24 hours) Kp,uu values of 0.03 in mice and 0.11 in rats after a 30 mg/kg p.o. dose. And approximately 10-fold greater than palbociclib. It can also increase the survival time of intracranial U87MG tumor-bearing rats similar to TMZ, and the combination of abemaciclib and TMZ was additive or greater than additive which turns out abemaciclib brain levels are reached more efficiently at presumably lower doses than palbociclib [42].
A phase 2 [NCT02308020] study of abemaciclib in patients with brain metastases secondary to hormone receptor-positive breast cancer has been launched. The primary objective was to evaluate intracranial objective response rate (iORR) in patients receiving abemaciclib with brain or leptomeningeal metastases (LM) secondary to hormone receptor-positive (HR+) metastatic breast cancer (MBC). However, although abemaciclib achieved therapeutic concentrations in brain metastases tissue, far exceeding those necessary for CDK4 and CDK6 inhibition, further studies are warranted [42].

Abemaciclib can also influence the cancer microenvironment enhancing innate immune activation via T cells and promoting the efficacy of PD-L1 checkpoint inhibitors [43]. A Phase I study (JPBJ, NCT02079636) of abemaciclib plus pembrolizumab, a programmed death receptor 1 (PD-1) antibody, demonstrated stable disease in 65% of pts with stage IV NSCLC along with a generally manageable safety profile and 14.3% initial ORR[44]. This study shows abemaciclib has a comparable potential for other cancer therapy except for breast cancers.

### 4.4 Dalpiciclib

Dalpiciclib (SHR6390), is a novel CDK4/6 inhibitor. In preclinical studies, dalpiciclib was shown to exert potent antitumor activity. SHR6390 exhibited potent antiproliferative activity against a wide range of human Rb-positive tumor cells in vitro, and exclusively induced G1 arrest as well as cellular senescence, with a concomitant reduction in the levels of Ser780-phosphorylated Rb protein. Compared with the well-known CDK4/6 inhibitor palbociclib, orally administered SHR6390 led to equivalent or improved tumor efficacy against a panel of carcinoma xenografts, and produced marked tumor regression in some models, in association with sustained target inhibition in tumor tissues. Furthermore, SHR6390 overcame resistance to endocrine therapy and HER2-targeting antibody in ER+ HER2+ breast cancer, respectively. Moreover, SHR6390 combined with endocrine therapy exerted remarkable synergistic antitumor activity in ER+ breast cancer [45]. This study results lead to the first in human phase I study in Chinese patients. In this open-label, phase 1 study, Chinese patients who had failed standard therapy were enrolled to receive oral dalpiciclib in 3 + 3 dose-escalation pattern at doses of 25-175 mg. Dalpiciclib showed an acceptable safety profile and dose-dependent plasma exposure in Chinese patients with ABC. In this phase 1 study, dalpiciclib showed an acceptable safety profile and dose-dependent plasma exposure in Chinese patients with ABC, which supports further phase 2 and phase 3 validation [46].

In the single-arm, phase 2 study [NCT04293276], HER2+ advanced breast cancer patients who had received no more than 1 line of systemic therapy in an advanced setting were recruited. Prior CDK4/6 inhibitors and HER2-targeted TKI were not allowed. Eligible patients received dalpiciclib 125 mg daily for 3 weeks and 1 week off, and pyrotinib 400 mg daily in 28-day cycles. The primary endpoint was the objective response rate (ORR) as per RECIST 1.1. 24 pts were enrolled in the first stage: HR+ disease, 54.2% (13/24); trastuzumab -treated, 66.7% (16/24); visceral metastasis, 91.6% (22/24). As of April 13th, 2021, of 23 evaluable patients 65.2% (15/23) had achieved confirmed ORR (15PR, 6SD, 2PD). 62.5% (15/24) of patients experienced tolerable grade 3/4 adverse events (AEs).

DAWNA-1 (NCT03927456), a double-blind, randomized, phase 3 trial of dalpiciclib plus fulvestrant in hormone receptor-positive, HER2-negative ABC with disease progression after endocrine therapy. A total of 361 patients were randomized 2:1 to receive dalpiciclib plus fulvestrant or placebo plus fulvestrant. The study met the primary endpoint, showing significantly prolonged investigator-assessed progression-free survival with dalpiciclib plus fulvestrant versus placebo plus fulvestrant (median = 15.7, 95% confidence interval (CI) = 11.1-not reached versus 7.2, 95% CI = 5.6-9.2 months; hazard ratio = 0.42, 95% CI = 0.31-0.58; one-sided p< 0.0001 (boundary was p≤ 0.008)) compared with 4.6 to 12.8 months in the placebo plus fulvestrant groups in trials of other
CDK4/6 inhibitors. The most common grade 3 or 4 adverse events with dalcipiclib plus fulvestrant were neutropenia (84.2%) and leukopenia (62.1%). The incidence of serious adverse events was 5.8% with dalcipiclib plus fulvestrant versus 6.7% with placebo plus fulvestrant [47]. According to DAWNA-1 data, NMPA approved dalcipiclib combined with fulvestrant in patients with recurrent or metastatic breast cancer who are hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, and progress after endocrine therapy. Currently, dalcipiclib is being investigated in many other cancer patients in clinical trial, such as recurrent/metastatic head and neck squamous cell carcinoma (NCT05724355), advanced/metastatic colorectal cancer (NCT05480280), luminal B/HER2-negative breast cancer (NCT05640778), nasopharyngeal carcinoma (NCT05724355).

4.5 Trilaciclib

Myelosuppression, or bone marrow suppression, is defined as a decrease in the ability of the bone marrow to produce blood cells. Myelosuppression may occur when the stem cells in the bone marrow are damaged (such as by chemotherapy drugs), when it is crowded (by tumor cells or fibrosis), or due to bone marrow failure. Chemotherapy is the basic treatment of tumor patients, and bone marrow suppression is the most common hematologic toxicity caused by traditional chemotherapy drugs. Chemotherapy-induced myelosuppression can be managed with supportive treatments such as hematopoietic growth factors and transfusions. However, these interventions are lineage-specific, administered after signs and symptoms of myelosuppression appear and are associated with their own adverse reactions. The utility of CDK4/6 blockade in this clinical setting relies on the fundamental dependence of hematopoietic cells on CDK4/6 as a regulator of normal progenitor replication and the near universal independence from CDK4/6 signaling in SCLC owing to the typical loss of RB1 gene in SCLC tumor biology. With the administration of trilaciclib, hematopoietic cells are transiently arrested in the G1 phase of cell cycle, and therefore potentially saved from toxicity from chemotherapy.

Trilaciclib is a short-acting, highly selective, reversible cyclin-dependent kinase 4/6 (CDK4/6) inhibitor being developed to reduce chemotherapy-induced multi-lineage myelosuppression, which inhibits the phosphorylation of Rb and induces an exclusive, reversible G1 arrest. In vitro and in vivo, trilaciclib protects Rb-competent cells from damage by chemotherapy and regulates the proliferation of HSPCs in both mouse and canine bone marrow, in a reversible, dose and time-dependent manner. Trilaciclib is currently the world's first and only drug with a comprehensive bone marrow protection effect. In February 2021, trilaciclib received its first approval in the USA to decrease the incidence of chemotherapy-induced myelosuppression in adult patients when administered before a platinum/etoposide-containing regimen or topotecan-containing regimen for extensive-stage small cell lung cancer (ES-SCLC). The recommended dose of trilaciclib is 240 mg/m$^2$ per dose to be administered as a 30-minute intravenous infusion completed within 4 h prior to the start of chemotherapy on each day chemotherapy is administered.

The approval is based on the results of three randomized, double-blind, placebo-controlled, pivotal phase 2 studies (Study 1:G1T28-05; Study 2: G1T28-02; Study3: G1T28-03) conducted by trilaciclib in patients with ES-SCLC and combined data analysis showed that 125 and 120 patients received an intravenous infusion of trilaciclib or placebo before chemotherapy[48]. The results found that 11.4% and 52.9% of patients with severe neutropenia in the trilaciclib group and placebo group respectively. The incidence of grade 3/4 anemia in the trilaciclib and the placebo groups was 20.3% vs 31.9%. Trilaciclib and placebo group had a grade 3/4 thrombocytopenia in 19.5% and 36.1%, respectively, and trilaciclib had a significant multilineage bone marrow protection effect. At the same time, the progression-free survival (5.3 months vs 5.0 months, p=0.1404) and the overall survival (OS: 10.5
months vs. 10.6 months, p=0.8316) in the trilaciclib group were similar to those in the placebo group, suggesting that trilaciclib did not affect the antineoplastic efficacy of chemotherapy. The combination of trilaciclib + chemotherapy is expected to solve the problem of bone marrow suppression, which has plagued doctors and patients for many years, and allow tumor patients to live longer and better [49].

Trilaciclib is also being investigated in other phase 2 and 3 trials for colorectal cancer (NCT04607668), triple-negative breast cancer (NCT04799249), non-small cell lung cancer (NCT04863248) and bladder cancer (NCT04887831). Several other novel agents: plinabulin, romiplostim, ALRN6924, and roxadustat are currently in clinical development for the prevention or treatment of multilineage or single-lineage myelosuppression in patients with various tumor types. The availability of treatments that could enable patients to maintain standard-of-care chemotherapy regimens without the need for additional interventions would be valuable to physicians, patients, and health systems.

Table 1: CDK4/6 inhibitors in clinical application

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<tr>
<th>Drug</th>
<th>Palbociclib</th>
<th>Ribociclib</th>
<th>Abemaciclib</th>
<th>Dalpiciclib</th>
<th>Trilaciclib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing route</td>
<td>O.P.</td>
<td>O.P.</td>
<td>O.P.</td>
<td>O.P.</td>
<td>I.V</td>
</tr>
<tr>
<td>IC50</td>
<td>CDK4 (D1): 11 nmol/L</td>
<td>CDK6:10 nM</td>
<td>CDK4:2 nM</td>
<td>CDK4:12.4 nM</td>
<td>CDK4 (D1): 1 nM;</td>
</tr>
<tr>
<td>Candidates</td>
<td>1. Indicated for the treatment of adults with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer. 2. Use in combination with an aromatase inhibitor as initial endocrine-based therapy for the treatment of postmenopausal women with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-positive advanced or metastatic breast cancer, as initial endocrine-based therapy. 3. Patients with disease progression following endocrine therapy. 4. Patients with hormone receptor (HR)-positive advanced or metastatic breast cancer, as initial endocrine-based therapy.</td>
<td>1. in combination with fulvestrant for patients with HR-negative, HER2-positive, HER2-negative advanced or metastatic breast cancer who are hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, and have progressed after endocrine therapy. 2. Monotherapy for women and men with HR-positive, HER2-negative advanced or metastatic breast cancer with disease progression following endocrine therapy. 3. in combination with fulvestrant for postmenopausal women with HR-positive, HER2-negative advanced or metastatic breast cancer, as initial endocrine-based therapy. 4. in combination with an aromatase inhibitor as initial endocrine-based therapy for postmenopausal women with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer.</td>
<td>1. Prevent chemotherapy-induced myelosuppression in ESCLC (extensive-stage small cell lung cancer (ES-SCLC)). Approved to decrease the incidence of chemotherapy-induced myelosuppression in adults when administered prior to a platinum/etoposide-containing or topotecan-containing regimen for ES-SCLC.</td>
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5. Combination therapy with CDK4/6 inhibitors

Over the past 20 years, cyclin-dependent kinases (CDKs) inhibitors have made great strides. Selective CDK4/6 inhibitors can block cell cycles from G1 to S, and clinical studies have shown good efficacy and fewer adverse reactions. In addition to combine with endocrine therapy, CDK4/6 inhibitors can be used in combination with chemotherapy, radiotherapy, PI3K/ mTOR inhibitors, immunotherapy, molecularly targeted drugs, and other therapies to overcome their drug resistance and improve clinical efficacy. This part reviews some of the combination therapy methods with CDK4/6 inhibitors.

5.1 Chemotherapy

Chemotherapy remains the main treatment option for patients with several tumor types. However, the utility of combining CDK4/6 inhibitors with standard chemotherapy remains unclear. Since chemotherapy targets only dividing cells, it has been argued that CDK4/6 inhibitors, by arresting cell proliferation in G0/G1 phases of the cell cycle, might protect tumor cells from the cytotoxic effects of chemotherapeutic compounds. Consistent with this notion, several studies in breast cancer cell lines, xenografts, and GEMM models documented that co-administration of CDK4/6 inhibitors antagonized the therapeutic effects of various classes of chemotherapeutic compounds that act during DNA synthesis (doxorubicin, gemcitabine, methotrexate, and mercaptopurine) or mitosis (taxanes) and even promoted multidrug resistance (MDR) occurrence, an effect counteracted by both CDK4 siRNA and palbociclib treatment[50, 51] [52]. While other preclinical and clinical studies show contrary results regarding the benefit of combining CDK4/6 inhibitors with standard cytotoxic chemotherapy. Palbociclib, ribociclib, and abemaciclib have been shown to enhance chemotherapy cytotoxicity when combined with camptothecin, carboplatin, cisplatin, docetaxel, doxorubicin, 5-FU, gemcitabine, irinotecan, paclitaxel, and temozolomide in RB-proficient in vitro and in vivo models of non-small cell lung carcinoma (NSCLC), ovarian cancer, gastric cancer, TNBC, atypical teratoid rhabdoid tumors, Ewing sarcoma, pancreatic cancer, and glioblastoma using both sequential and
concurrent dosing schedule[53]. Thus, even if CDK4/6 inhibitors are found to improve the antitumor effects of chemotherapy, the ability to circumvent the overlapping toxicity of bone marrow suppression will remain a challenge in the clinic.

5.2 Radiotherapy

Up to now, no definite evidence regarding the safety and efficacy of the combination of CDKIs plus radiotherapy (RT) is currently available. Several preclinical studies have proved the possible synergy between CDK4/6i and radiotherapy for many types of tumors in vitro and in vivo [54]. Besides, repression of CDK4 by the NFATc1 transcription factor appears to be involved in the maintenance of quiescence in hair follicle stem cells [55]. Moreover, radiotherapy-resistant cancer stem cells isolated from liver cancer and glioblastoma cell lines demonstrated upregulation of AKT and cyclin D-CDK4 signaling; inhibition of AKT, cyclin D, or CDK4 resulted in re-sensitization to radiotherapy in these cells [56]. The mechanism of CDK4/6 inhibitor radiosensitization may be related to the induction of cancer stem cell differentiation, which increases the vulnerability of cells to radiation-induced cell death [57].

In most of the above studies, CDK4/6i was started at the same time as radiotherapy or following it. However, several studies have focused on determining which sequence of the combination was most valuable. The previously cited studies by Petroni et al. and Hashizume et al. compared in vitro and in vivo efficacy of several treatment sequences [58, 59]. In both studies, the sequence of radiotherapy followed by palbociclib (respectively 6 h and 12 h after) offered a superior cytostatic effect to all the other sequences (concomitant radiotherapy and palbociclib, palbociclib followed by radiotherapy, radiotherapy alone and palbociclib alone). It is noteworthy that the 14-day palbociclib followed by the radiotherapy sequence provided worse local control and survival than radiotherapy alone [58]. In mice xenografted with ATRT or glioblastoma cell lines, radiotherapy (5 × 1 Gy daily) followed by 14 days of palbociclib and the combination of radiotherapy (5 × 1 Gy) and palbociclib delivered concomitantly with a continuation of palbociclib for a total of 14 days offered both the best local control and survival, but the difference with radiotherapy alone was not significant [59]. The possible clinical benefits of CDK4/6 blockade on radiation sensitization need to be further explored.

5.3 PI3K/AKT/mTOR pathway

PI3K/AKT/mTOR signaling pathway is essential for cell proliferation, survival, and metabolism. Which is activated in approximately 30%-40% of breast cancers, particularly in the HR-positive subtype. Furthermore, the correlation of the PIK3/AKT/mTOR pathway with resistance to CDK4/6 inhibitors has also been reported recently in several tumors. In breast cancer, aberrant mTORC1 activation increased cyclin D1 overexpression, participating in CDK4/6 inhibitors resistance. In addition, the upregulation of both cyclin D1 and cyclin E was also observed in pancreatic cancer models that were sensitive to mTOR inhibitors [60]. These results suggest that the inhibition of the PI3K/AKT/mTOR pathway in combination with CDK4/6 inhibitors may have potential therapeutic benefits in overcoming resistance to CDK4/6 inhibitors as well as in augmenting anticancer activity in CDK4/6 inhibitor-sensitive settings.

Recently, a novel triple CDK4/6 and PI3K-δ kinase inhibitor ON123300 (IC50 CDK4/cyclin D1 3.81nm, PI3K-δ 144.4 nm) exhibit potent activity against ibrutinib-sensitive and resistant mantle cell lymphomas (MCLs) both in vitro and in vivo. ON123300 can induce cell cycle arrested in G0/G1 at lower concentrations, higher concentrations resulted in apoptosis [61]. The FDA has granted permission for a phase 1 study evaluating ON123300, a first-in-class multi-kinase CDK4/6 inhibitor the safety, tolerability, and pharmacokinetics in patients with relapsed/refractory advanced cancers, including hormone receptor (HR)-positive, HER2-negative breast cancer that is resistant to approved
second-generation CDK4/6 inhibitors, multiple myeloma, advanced hepatocellular carcinoma, and inoperable glioblastoma (NCT04739293). Further investigations will be necessary to evaluate the potential role of CDK4/6 and PI3K pathway inhibitor combinations for treating multiple kinds of advanced cancers and for determining biomarkers that are predictive of response.

5.4 Immune checkpoint blockade

Immune checkpoint blockades have shown promising efficacy for various cancers in recent years. However immune-related pathways are correlated with the emergence of resistance to various anticancer drugs, such as those of IFN-α and IFN-β, which were reported to be enriched in CDK4/6 inhibitor-resistant breast cancer cells. In many preclinical studies, CDK4/6 inhibitors were reported to promote anti-tumor immunity. Scientists from Dana-Farber Cancer Institute had confirmed that CDK4/6 inhibitors not only block cancer cell division but also stimulate the immune system to attack and kill cancer cells. CDK4/6 inhibitors trigger antitumor immune responses mainly in two ways. In cancer cells, the drugs caused a huge increase of abnormal proteins on the tumor cell surface. These proteins, called antigens, can act as signals from the immune system that diseased or cancerous cells are present and need to be removed. The drugs also reduced the number of T regulatory cells (Tregs)[62]. Moreover, other teams had showed that CDK4/6 inhibitor monotherapy induces intra-tumor T cell inflammatory signature and treatment causes MHC class I and 2 upregulation in tumor cells and increased NFAT signaling in T cells[63]. Besides, CDK4/6 could improve immunotherapy response and promotes chemokine-mediated T-cell tumor homing which attracts T cells to invade the tumor, thus inhibiting tumor growth more effectively [43]. Taken together, CDK4/6 inhibition potentiates anti-tumor immunity and enhances the response to PD-1 blockade, providing a rationale for new anti-cancer therapeutic strategies combining CDK4/6 inhibitors with immunotherapies. Although many preclinical experiments have demonstrated the effectiveness of the combination of PD-1/L1 and CDK4/6, these trials have largely been stopped due to severe side effects in clinical trials of early and advanced hormone-receptor-positive breast cancer [64]. Given the failure of clinical trials of CDK4/6 inhibitors in combination with PD-1 blockers, another study suggests that the greatest benefit of CDK4/6 inhibitors may occur in early treatment, that is, early use of CDK4/6 inhibitors to establishing memory CD8 T cells, followed by immune checkpoint blockade, may avoid synergistic toxicity when CDK4/6 inhibitors are used in conjunction with PD-1 blockers. This therapy is currently being explored in clinical trials (NCT04075604), which may pave the way for cancer treatment in the future.

6. Conclusion

Due to their important roles in regulating the cell cycle, CDKs are promising targets for the development of the anticancer drug, especially CDK4/6. Until now, five CDK4/6 inhibitors have been approved by the FDA and CFDA for the treatment of breast cancer and as protective agent for bone marrow suppression. Based on the excellent therapeutic effect and good safety of CDK4/6 inhibitor combined with endocrine therapy, it has become the standard regimen recommended by domestic and foreign guidelines for the first-line treatment of HR+/HER2- advanced breast cancer. However, issues resulting from resistance to CDK4/6 inhibitors are emerging. Evidence collected from preclinical and clinical studies has suggested that various cell-specific and non-cell specific mechanisms may contribute to intrinsic or acquired resistance to CDK4/6 inhibitors. Therefore, further investigations are warranted for both mechanistic and clinical validation to define more precise mechanisms of resistance to CDK4/6 inhibitors, and to develop successful therapeutic strategies to overcome resistance. An important challenge will be to test and identify combinatorial treatments targeting upstream and downstream pathways that have synergistic effects to overcome drug resistance.
CDK4/6 inhibitors for the treatment of different tumor types in clinical trials.

CDK4/6 inhibitors trigger cell cycle arrest of tumor cells and, in some cases, senescence. It will be essential to identify combination treatments that convert CDK4/6 inhibitors from cytosstatic compounds to cytotoxic ones, which would unleash the killing of tumor cells. Besides, current CDK4/6 inhibitors were designed to targeting the ATP binding pocket, and the amino acid sequences of the ATP-binding pockets they bind have 94% sequence consistency. Lack of selectivity can have serious toxic side effects, limiting its therapeutic window. However, the revolutionary proteolysis-targeting chimeras (PROTACs) technology offers a novel method of dealing with this. PROTACs have greater selectivity than the authorized CDK4/6 inhibitors by specifically inducing the degradation of CDK6 while having no impact on CDK4 in the proteome range[65]. The limitation of PROTACs is whether it is possible that they can be used as drugs for patients since PROTACs have trouble crossing cell membranes. However, as more and more candidate drugs based on PROTAC technology have entered clinical trials, Future research should focus more on CDK4/6 protein degraders because they are likely to have better efficacy, fewer side effects, and show no resistance. Above all, more and more CDK4/6 inhibitors will be developed and tested during the next years.

Acknowledgement

The author has made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; She has drafted the work or revised it critically for important intellectual content; the author has approved the final version to be published; she agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

[62] Goel, S., et al., CDK4/6 inhibition triggers anti-tumour immunity, (1476-4687 (Electronic)).