Annals of *Research* in *Oncology*
Vol. 6(2), 60-81, 2026

REVIEW

CYCLIN-DEPENDENT KINASES: MECHANISMS OF ACTION AND THERAPEUTIC POTENTIAL

Sivan **Brodo-Abo** [1], Vrunda **Satasiya** [1, 2], Giulio **Mazzaroti** [1, 2], Shendi **Shani** [2, 3], Andrea **Morrione** [1, *], Antonio **Giordano** [1, 2, *]

[1] Sbarro Institute for Cancer Research and Molecular Medicine Center for Biotechnology, Department of Biology, College of Science and Technology, Temple University, Philadelphia (PA), U.S.A.
[2] Department of Medical Biotechnologies, University of Siena, Siena, Italy
[3] S.H.R.O. Italia Foundation ETS, Candiolo, Turin, Italy
[*] Correspondence to: andrea.morrione@temple.edu; https://orcid.org/0000-0002-2319-7884;
antonio.giordano@temple.edu; https://orcid.org/0000-0002-5959-016X

ABSTRACT: Cyclin-dependent kinases (CDKs) are critical regulators of the cell cycle and play essential roles in DNA repair and maintenance of genome stability. Importantly, dysregulation of CDK activity is a key contributor to oncogenesis. CDKs functionally interact with various regulatory molecules, including cyclins, CDK protein inhibitors, and transcription factors such as p53 and retinoblastoma (RB) proteins. Upon mitogenic stimuli, CDK4/6 binds to cyclin D proteins, leading to hyperphosphorylation of RB proteins, activation of E2F-dependent transcriptional programs, as well as cell cycle initiation and progression. Dysregulation of CDK4/6 action is associated with cancer, and CDK4/6 inhibitors have emerged as very important anticancer therapies. This review will focus on the molecular mechanisms by which CDKs and cyclins regulate the cell cycle, their roles in maintaining genomic integrity, their interactions with RB, and the therapeutic potential of CDK 4/6 inhibitors.

Doi: 10.48286/aro.2026.124

Impact statement: The transition from broad-spectrum kinase inhibitors to selective CDK4/6 inhibitors represents an important step in oncology, moving away from high-toxicity "pan-CDK" approaches toward targeted, precision medicine.

Key words: *CDKs; Cyclins; CDK inhibitors; RB Proteins; Cell cycle.*

Received: Feb 10, 2026/**Accepted:** Apr 09, 2026

Published: Jun 30, 2026

INTRODUCTION

Cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases with crucial roles in many cellular processes, including cell cycle progression and transcriptional regulation (1). CDKs were originally identified and categorized based on their functions in cell cycle regulation and their association with activating partners, such as cyclins and CDK activating kinases (2-4). CDKs involved in cell cycle regulation include CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK14, CDK15, CDK16, CDK17, and CDK18. Whereas CDK7, CDK8, CDK9 (5), CDK10, CDK11, CDK12, CDK13, CDK19, and CDK20 are involved in transcriptional regulation. CDK2, CDK4 and CDK7 play a role in immune response activation, while CDK1, CDK2, CDK5 and CDK12 contribute to the DNA damage response (DDR). Accordingly, CDK activity is tightly regulated in a cell cycle phase-dependent manner by specific cyclins, changes in phosphorylation, and other regulatory molecules including CDK protein inhibitors (6). Beyond their key role in triggering cell cycle entry (**Figure 1**), CDKs orchestrate DNA synthesis and chromosome segregation (7), which are crucial processes for cell cycle progression and cell fate determination.

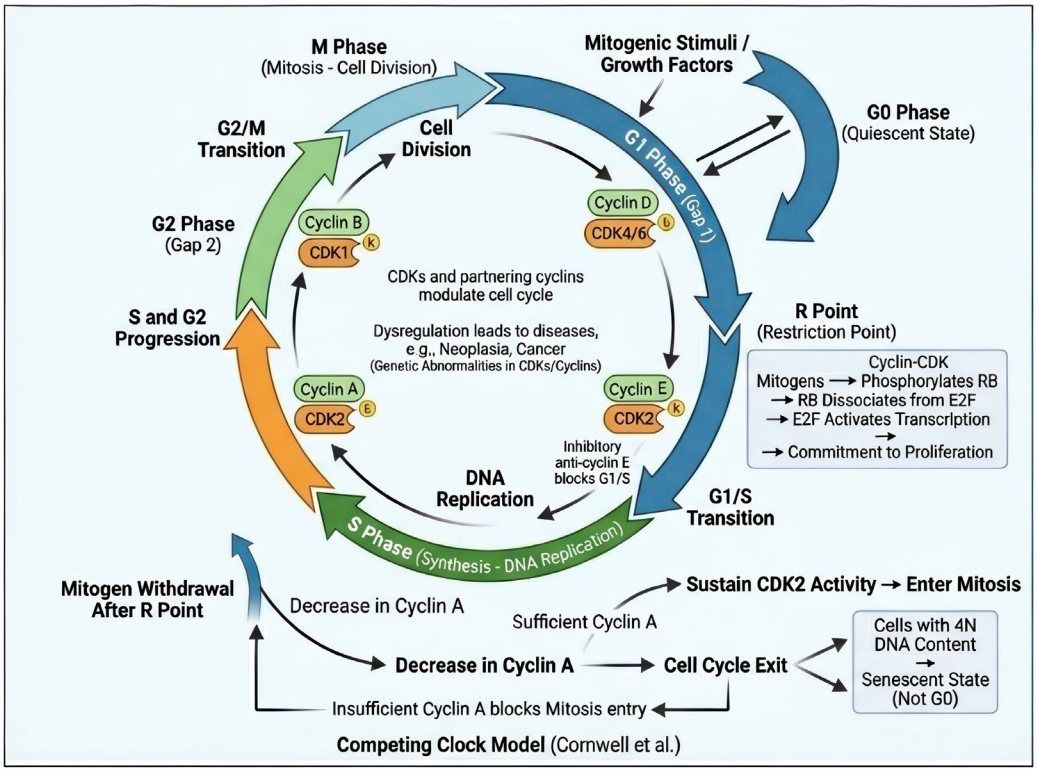

Figure 1. *Overview of cell cycle regulation and the revised "Competing Clock" model.*
This diagram illustrates the major phases of the mammalian cell cycle (G1, S, G2, M) and the quiescent state (G0). Cycle progression is driven by mitogenic stimuli and governed by specific CDK and cyclin complexes (e.g., Cyclin D-CDK4/6, Cyclin E-CDK2). The canonical Restriction Point (R Point) mechanism is detailed on the right. Cyclin-CDK-mediated phosphorylation of RB proteins releases the E2F transcription factor, leading to commitment to proliferation. The lower section presents the revised "Competing Clock Model" (Cornwell et al., 2023). Contrary to the canonical view of irreversible commitment after the R point, this model proposes that mitogen withdrawal post-R point leads to a decrease in Cyclin A levels. Insufficient Cyclin A fails to sustain necessary CDK2 activity, resulting in a cell pool exiting cycle in a senescent state with 4N DNA content, rather than entry into mitosis.

CDK4 and CDK6 (CDK4/6) are the main CDKs responsible for cell cycle initiation and proliferation, as they modulate retinoblastoma (RB) family tumor suppressor protein activity by promoting their hyperphosphorylation (8). Hyperphosphorylated RBs are inactivated, thereby releasing E2F-dependent transcriptional programs (9-11). Dysregulation of CDKs, specifically (CDK4/6), are associated with cancer initiation. Thus, CDK4/6 inhibitors are very attractive targets for cancer therapy. In this review, we will discuss molecular properties of CDKs and cyclins, including their main protein interactors, role in modulating the cell cycle, and focus on CDK4/6 inhibitors currently used in clinical practice. Although CDK4/6 inhibitors have transformed the therapeutic landscape of hormone-receptor-positive (HR^+) breast cancer, substantial unmet needs remain, including mechanisms of drug resistance, incomplete understanding of CDK4 *versus* CDK6 dependency across tumor types, lack of robust predictive biomarkers, and limited benefit in RB-deficient or Triple-Negative Breast Cancer (TNBC). Moreover, the expanding evidence that CDK inhibition modulates tumor immunogenicity underscores the need to redefine CDK targeting beyond cytostatic control and toward rational combination strategies. Thus, a comprehensive reassessment of CDK biology and therapeutic targeting is both timely and necessary.

THE CELL CYCLE

The cell cycle is crucial for controlling cell development and cell regulation (12). The cell cycle, as shown in **Figure 1**, is divided into G0, G1, S, G2, and M phases. When cells are withdrawn from the cell cycle, they are in a quiescent state, defined as the G0 phase (13). The S and M phases are parted by the G1 and G2 phases, which are defined also as "Gap Phases." Upon mitogenic stimuli, cells enter

the cell cycle in the G1 phase and then proceed through the synthesis (S) phase, where DNA replicates, followed by the G2 phase (13). In the mitosis (M) phase, cell division occurs. CDKs and partnering cyclins are essential components in modulating the cell cycle (**Figure 1**), and dysregulation of this process is associated with many diseases, including neoplasia. Genetic abnormalities such as gene amplification (*i.e.*, *CCND1, CDK4*), overexpression (*i.e.*, Cyclin E), translocation (*i.e.*, t11;14), and 3'-UTR mutations can often cause dysregulations in CDKs, cyclins, affecting cell cycle progression and possibly leading to cancer development (12, 14-16).
In mammalian cells, growth factor stimulation promotes cell exit from the G0 phase and initiation of the G1 phase (2). Importantly, CDK4/6 interaction with cyclin D is the primary contributor to starting the G1 phase. Furthermore, the activation of CDK2 and cyclin E begins at the G1/S phase transition, as confirmed by using inhibitory anti-cyclin E antibodies, which blocked the cells at the start of the G1/S phase transition (17, 18).
The restriction (R) point of the cell cycle is a crucial checkpoint in late G1 when cells commit to proliferation and DNA replication at the G1/S transition and become largely independent of continued mitogenic stimulation (18). In early G1, mitogen-dependent induction of D-type cyclins promotes the formation of cyclin-D-CDK4/6 complexes, which initiate RB phosphorylation and weaken RB-mediated repression of E2F transcription factors (2, 19). As mitogens promote the assembly of cyclin-CDK complexes, they activate cyclin-CDKs, which progressively phosphorylate RB, destabilizing inhibitory RB-E2F complexes and releasing E2F proteins to activate transcription of genes required for S-phase entry (9, 20). Consistent with this sequential model, cyclin E-CDK2 activity rises at the G1/S transition, further reinforcing RB inactivation through RB hyperphosphorylation and establishing a CDK-E2F positive feedback loop that consolidates commitment to S phase (9, 17).
Before passage through the R point, mitogen withdrawal reduces upstream cyclin D-CDK4/6 signaling and favors re-establishment of RB-dependent repression, in part through increased CDK inhibitor activity (*e.g.*, p21/p27), thereby preventing RB hyperphosphorylation and blocking E2F-dependent transcription (21, 22). By contrast, after cells pass the R point, RB remains functionally inactivated and the E2F-driven transcriptional program sustains progression through S phase even when mitogenic signals decline, while subsequent cyclin-CDK complexes coordinate orderly transitions across the remaining phases of the cell cycle (**Figure 1**) (18).
Cornwell and colleagues recently presented a revised model, the competing clock model, in which they proposed that mitogen withdrawal results in a decrease of cyclin A protein level resulting in cell cycle exit, even after passing the R point (18). Notably, cells with 4N DNA content exiting the cell cycle (about 15%) are not in G0 but in a sort of senescent state. Cells enter mitosis only when sufficient cyclin A protein is available to sustain CDK2 protein activity (18).

CDKS

CDKs are conserved across eukaryotes, ranging from unicellular organisms such as yeast to complex mammalian organisms (23). In humans, the CDK family comprises 20 members classified into two main categories: "cell cycle CDKs" and "transcriptional CDKs" (24-26). The "cell cycle CDKs" are CDK1, CDK2, CDK3, CDK4, CDK6, and CDK7, and primarily regulate cell cycle progression, whereas the "transcriptional CDKs", CDK7, CDK8, CDK9, first identified as proteins containing the "PITALRE" motif (27), CDK10, originally cloned as a "PISSLRE"-containing protein (27, 28) and CDK11, CDK12, and CDK13, modulate the transcription of specific target genes (24).
All CDK proteins share a conserved 250 amino acid catalytic domain with two-lobe structures: the amino-terminal (N) lobe and the carboxy-terminal (C) lobe (29). The N lobe presents β-sheets while the carboxy-terminal lobe presents α-helices and has an active sandwich-like site in between. The N-lobes have a glycine-rich inhibitory element, known as the G-loop, while C-lobes have an activation segment which contains the conserved PSTAIRE sequence in CDK1. The C-lobes also contain an activation domain, which extends from the Asp-Phe-Gly (DPG) motif to the Ala-Pro-Glu (APE) motif (30), while including phosphorylation-sensitive residues in their T-loops (31). For all CDKs except CDK5, phosphorylation of T-loops is required for kinase activation.
CDK4/6 are homologous CDKs that are essential for mammalian cell proliferation (19), as they drive progression through the cell cycle from the G1 phase to S phase. CDK4/6 function differs from CDK1 and CDK2, which work during later stages of the cell cycle. The activity of CDK4/6 is tightly regulated by cyclin D isoforms (D1, D2, and D3), whose expression is modulated by extracellular signals, including mitogens, cytokines, and cell-cell contact (19, 32). Under mito-

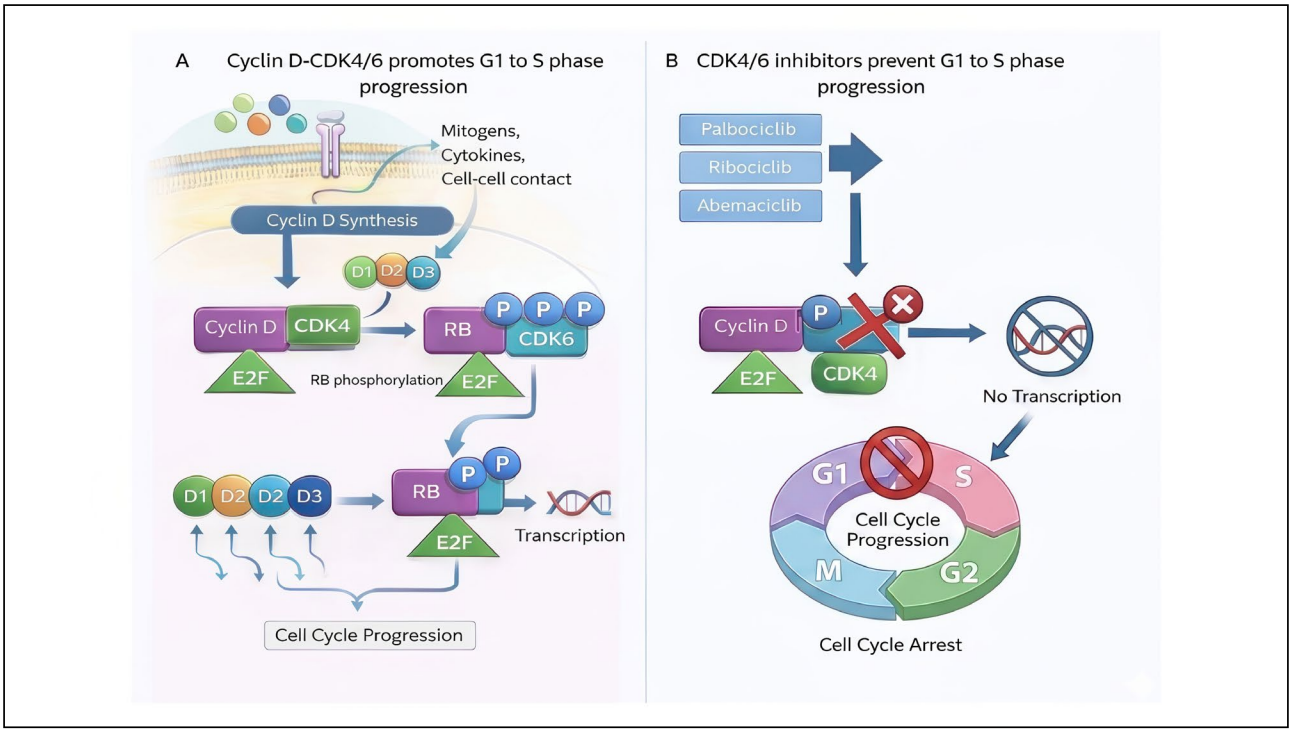

Figure 2. *Regulation of G1-S phase transition by Cyclin D-CDK4/6 and selective CDK4/6 inhibitors action.*
(A) *Mitogenic signals, including growth factors, cytokines, and cell–cell contact, induce cyclin D during early G1 phase. Cyclin D isoforms (D1, D2, and D3) associate with CDK4 and CDK6 to form active Cyclin D-CDK4/6 complexes, which phosphorylate RB proteins. RB phosphorylation disrupts its inhibitory action on E2F transcription factors, resulting in E2F activation and transcription of genes required for G1-S phase transition and cell cycle progression.* ***(B)*** *Pharmacological inhibition of CDK4/6 by selective inhibitors, including palbociclib, ribociclib, and abemaciclib, block Cyclin D-CDK4/6 kinase activity and prevents RB phosphorylation. Thus, RB remains bound to E2F, suppressing E2F-dependent transcription and leading to G1 cell cycle arrest, thereby preventing progression into S phase.*

genic stimuli, D-type cyclin levels increase, leading to the formation and activation of cyclin D-CDK4/6 complexes and subsequent phosphorylation and inactivation of RB proteins, releasing their inhibitory action on E2F transcription factors, thereby promoting transcription of genes essential for cell cycle progression and entry into S phase (**Figure 2A**). It is important to specify that CDK7 plays a dual role within the cell. In addition to being a crucial component of the general transcription factor TFIIH, it functions as a Cyclin-Activating Kinase (CAK). In this capacity, CDK7 phosphorylates the T-loop activation segment of several key kinases, including CDK1, CDK2, CDK4, and CDK6, thereby enabling their catalytic activity (33).

CYCLINS

Cyclins are key for cell cycle progression and work with their cell cycle-specific CDK partners (34). The cyclin-CDKs complexes are essential modulators of the cell cycle, each complex working at specific phases of the cell cycle. Cyclin proteins contain cyclin box domains (CBDs) or CBD-like domains which regulate cyclin-CDK binding (35). CBDs were first discovered in the cell cycle proteins of sea urchins. There are 46 human proteins which contain CBDs or CBD-like domains, where 29 of them are direct CDK activators, such as Speedy A and p35 (35). However, not all CBDs and CBD-like domains-containing cyclins have a CDK partner, like G-type cyclin.

D-type cyclins are crucial for controlling cell cycle transition to the G1 phase as well as cell cycle progression (36) through the G1/S transition phase. D-type cyclins are essential, especially during mitogenic signaling, where they are required for initiating cell cycle in response to growth factor stimulation. D-type cyclins form a complex with CDK4 or CDK6 to modulate the transition from the G1 phase to S phase. D-type cyclins are essential prognostic biomarkers, especially for neoplasia since they are predictors of cell proliferative capacity (34). Three genes encode for specific D-type cyclins, each located

on a different chromosome (36). Those genes are *CCND1:*11q13.3, *CCND2:*12p12.23, and *CCND3:* 6p21.1, which encode for a specific D-type cyclin that share approximately 57% protein sequence identity and are expressed during the G1 phase and G1/S phase of the cell cycle.

During S phase, the cyclin A-CDK2 complex promotes DNA replication and facilitates the S to G2 transition (37). As cells progress into late S/G2, cyclin A increasingly associates with CDK1, supporting early mitotic events and faithful chromosome segregation, in part by regulating the stability of kinetochore-microtubule attachments (37).

Eukaryotic species have three B-type cyclin genes (*CCNB1*, *CCNB2*, and *CCNB3*), which are expressed during the G2/M phase transition period of the cell cycle (38, 39). In yeast model organisms, the Mcm1 transcription factor interacts with Swi Five Factor (SFF), and SFF mediates cell cycle-dependent promoter activation of *CCNB1* and *CCNB2*. In addition, the *CCNB1* promoter is regulated by *cis*-acting elements, which require the activation of a G2/M promoter; therefore, cyclin B works with CDK1 for activation of the G2/M transition period, indicating a phase-specific CDKs activation, and expression of their accompanying cyclins, including regulation during the transition phase (38).

E-type cyclins are also essential proteins during the G1 and G1/S transition phase of the cell cycle (40, 41). Mammals express two E-type cyclins, cyclin E1 and cyclin E2, which activate CDK2 to promote cell cycle progression via phosphorylation of cellular proteins during the G1 and S phases. Cyclin E1 and cyclin E2 are expressed in all proliferating cell types and share similar amino acid sequences. In addition, E-type cyclins can also activate CDK1 and CDK3 proteins, and elevated cyclin E-CDK2 activities were observed in various human tumors, as they are correlated to anticancer drug resistance (41). However, in normal growth conditions, the cyclin E-CDK2 complex is most active in cells that are in the G1 phase (40).

Cyclin M (FAM58A), in association with CDK10, regulates transcription by phosphorylating ETS2, which in turn modulates the expression of targets such as c-RAF. Scientific evidence highlights the role of CDK10 in the G2/M transition and in maintaining genome stability. Clinically, the loss of function of the cyclin M/CDK10 complex has been linked to endocrine therapy resistance in breast cancer, specifically contributing to the mechanisms of tamoxifen resistance (33). Low levels of the Cyclin M/CDK10 complex are associated with a broad form of endocrine resistance in breast cancer extending beyond tamoxifen to other estrogen receptor (ER)-targeted therapies, including aromatase inhibitors and fulvestrant (42). Loss or reduced expression of CDK10 promotes activation of alternative proliferative signaling pathways, most notably the RAF-MEK-ERK MAPK cascade, through stabilization of the transcription factor ETS2 and increased expression of c-RAF. Activation of this pathway enables tumor cell proliferation in an estrogen-independent manner, thereby diminishing their reliance on ER signaling for growth and survival. As a result, therapies inhibiting estrogen production or blocking ER activity become less effective. Consequently, low CDK10 expression has been proposed as a potential biomarker of poor response to endocrine therapies (43-45).

CELL DIVISION CYCLE 25 (CDC25)

CDC25 proteins are key members of the CDK phosphatase family and play an essential role in positively regulating cell division by activating CDKs (46-48). The CDC25 family includes three isoforms: CDC25A, CDC25B, and CDC25C (49). Results from immunofluorescent analysis showed that CDC25A localizes primarily in the nucleus, suggesting that it may be also important for DNA replication. Additionally, CDC25A is regulated throughout the cell cycle, and this protein plays a central role in regulating cell cycle progression, and it is especially active at the G1/S transition phase and in the M phase (28, 48). Its constitutive activity or overexpression can disrupt normal checkpoints and lead to oncogenesis (48). CDC25B exhibits dual specificities for phosphatase enzymes, which promote cell division by activating the cyclin B-CDK1 complex (49). CDC25B phosphatases dephosphorylate phosphotyrosine (pTyr15) and phosphothreonine (pThr14) residues on CDK1, which results in mitotic entry. CDC25C is active during the S phase and M phase (50). CDC25B begins activating cyclin B-CDK1 in the G2/M transition phase, but then the activation is completed by CDC25C at the start of the M phase (50). Furthermore, CDC25A is stabilized upon cyclin B-CDK1 phosphorylation, and creates a positive feedback loop with CDC25C, which finishes the activation of cyclin B-CDK1, which then leads to mitotic entry.

Although there is little knowledge about the functions of CDC25C, it was demonstrated that it can prevent progression to the S phase (50). CDC25A and CDC25B protein levels change in a cell-cycle-dependent man-

ner, while CDC25C protein levels remain constant throughout the cell cycle. Clinically, the *CDC25* gene was one of the ten most predominant genes associated with gastric cancer (GC) when mutated (49). The CDC25 proteins are also targets of the MYC transcription factor, which regulates their function (49).

RB PROTEINS

RB protein, RB1(p105), was identified over 30 years ago as the first human tumor suppressor protein (51-55). In addition to p105, the RB family includes RB-like RBL1(p107) and RBL2(p130), which all share structural similarities and function as tumor suppressor proteins. RBs are known as pocket proteins and consist of three main structural domains: the N-terminal domain, the A/B pocket, and the C-terminal domain (56, 57). Each domain has phosphorylation sites that are important for modulating p105 activities and mediating functional interactions with transcription factors and RB-binding proteins, such as p53. Published data indicated that p105 and p53 work in concert and are critical for cell growth arrest and apoptosis upon DNA damage (2). The direct physical interaction between p53 and Rb is limited to specific cellular contexts, but p53 and RB tumor suppressor pathways are well known to intersect through multiple regulatory mechanisms. One important point of convergence involves the DREAM (DP, RB-like, E2F and MuvB) complex, which contains the RB family members p107 and p130 and mediates p53-dependent transcriptional repression of a large set of cell cycle genes. Through this complex, p53 regulates hundreds of genes involved in cell cycle progression, DNA replication, and mitosis, thereby coordinating cell cycle arrest and maintaining genomic stability (58, 59). The N-terminal domain is essential for RB functions as a tumor suppressor, stabilization of p105, and phosphorylation-dependent regulation of RB activity (56, 57). The A/B pocket domain works as a binding site for a diverse array of viral and cellular proteins and plays key roles in maintaining the regulatory functions of p105. The C-terminal domain facilitates binding to the E2F-DP complex, which is critical for inhibiting cell proliferation and controlling the cell cycle, especially in the G1/S transition period (20).

In the G0 phase, p130 is the most abundant RB family member. After cells are stimulated to enter the cell cycle, p105 and p107 expression is induced, as they are E2F target genes. When expression levels of p105 and p107 increase, the expression levels of p130 decrease accordingly. In subsequent cell cycle phases, p105 and p107 expression levels remain constant while p130 levels are relatively low under these specific growth conditions (20). Pocket proteins, including p107 and p130, function as key regulators of cell cycle control and cellular fate decisions. During differentiation, these proteins act as molecular "brakes" enabling cells to exit the cell cycle and initiate specialized developmental programs, such as muscle or bone formation (60). Consistent with this role, p107 and p130 loss can lead to premature or defective differentiation as cells could fail to properly withdraw from proliferation, as observed in cartilage development. Pocket proteins also contribute to the regulation of cellular senescence by mediating cell cycle arrest. For example, p107 is required for the initiation of senescence in certain settings when p105 is absent. In addition, pocket proteins often promote cell survival by restraining the activity of E2F transcription factors, particularly E2F1, which can induce apoptosis. By inhibiting E2F activity, p107 and p130 help prevent E2F-driven cell death. This regulatory role is highlighted by studies showing that the simultaneous loss of all three pocket proteins (p105, p107, and p130) results in broad ectopic apoptosis in the brain. Although elevated levels of pocket proteins can facilitate certain forms of cell cycle-associated cell death in certain context, their predominant role during development is to maintain cellular homeostasis by preventing premature or inappropriate apoptosis (61). These pocket proteins were first identified as a homolog of p105 and target points for transforming domains of viral oncogenic proteins (62).

p105 tightly regulates the E2F transcription factors, which are key players in regulating cell proliferation (63). Among them, E2F1-E2F3 function primarily as transcriptional activators that stimulate the expression of genes required for S-phase entry, whereas E2F4 and E2F5 act predominantly as transcriptional repressors. p105 preferentially binds and inhibits the activation of E2Fs (E2F1, E2F2, E2F3a) to regulate the G1/S transition. Conversely, the p105 protein preferentially associates with E2F1-E2F5 transcription factors, while p107 and p130 form complexes exclusively with E2F4 and E2F5 (64). This interaction is crucial for maintaining cellular quiescence (G0) and preventing unscheduled reentry into the cell cycle. The p105 family members control different aspects of E2F activity, but the specific functions of different RB family members are not

yet well defined. To characterize the specific role of these pocket proteins, Hurford *et al*. (1997) investigated how the expression of E2F-regulated genes is affected in cells deleted of each of the RB family members (65). There were no differences detected in the expression of E2F-target genes in cells deleted of either p107 or p130, but dysregulated expression of E2F target genes was evident in cells deleted of p105 and in cells that were deleted of both p107 and p130. These results proved that p105, p107, and p130 play different roles in regulating E2F. Hurford *et al*. studied these RB proteins and discovered that cells with a triple knockout of p105, p107, and p130 had an increased expression of E2F target genes (65). Additionally, the p105/E2F, p107/E2F, and p130/E2F complexes are active repressors on E2F sites, each with different roles for E2F regulation. Thus, E2F sites are critical targets for p105 family proteins, which regulate cell proliferation (65).

Early studies investigating the specific roles of the RB pocket proteins, p105, p107, and p130, demonstrated that these proteins regulate E2F-dependent transcription through distinct yet overlapping mechanisms (66-69). In one of the early genetic analyses, Hurford *et al*. showed that deletion of individual RB family members had differential effects on the expression of E2F-regulated genes. Loss of either p107 or p130 alone had minimal impact on E2F target gene expression, whereas deletion of p105, or combined deletion of p107 and p130, resulted in dysregulated expression of E2F-responsive genes (65). Moreover, cells lacking all three pocket proteins exhibited a strong increase in E2F target gene expression, demonstrating that RB family members collectively repress E2F-dependent transcription and control cell-cycle progression (70).

Subsequent work expanded this model by identifying the DREAM complex (DP, RB-like, E2F, and MuvB) as a central regulator of cell-cycle gene repression in quiescent and early G1 cells (71, 72). Seminal studies from the laboratory of James DeCaprio demonstrated that p130 and p107 associate with E2F4/5 and the MuvB core complex to form the DREAM complex, which represses a large set of cell-cycle genes during quiescence (71). Later studies from Kurt Engeland and others further established that DREAM coordinates transcriptional repression of genes required for S phase and mitosis by binding to E2F and CHR promoter elements (73-75). Upon cell-cycle entry, phosphorylation of pocket proteins by cyclin-dependent kinases disrupts the DREAM complex, allowing the MuvB complex to transition into transcriptionally activating complexes such as MMB (Myb-MuvB) and FOXM1-MuvB, thereby promoting expression of genes required for DNA replication and mitosis (71, 76-78).

Collectively, these findings refined the earlier RB-E2F model by demonstrating that RB proteins function within larger multiprotein complexes that dynamically regulate cell-cycle gene expression. In particular, p130-containing DREAM complexes play a dominant role in maintaining transcriptional repression during cellular quiescence, whereas p105 primarily regulates E2Fs during the G1/S transition. This integrated regulatory network ensures tight control of cell proliferation through coordinated repression and activation of E2F target genes.

p105 is frequently inactivated in a wide range of tumors, and *RB1* loss or functional mutations are associated with pediatric RB cancers (4, 56). Mutations or deletions in the *RB1* gene can lead to uncontrolled cell proliferation, thereby promoting cancer initiation (4).

p105 interacts with the adenovirus early region 1A (E1A) oncoprotein in adenoviruses (79). The binding between p105 and E1A prevents p105 interactions with E2Fs, thereby inducing cell proliferation and replication of adenoviral DNA (80). p105 binds to E1A via the LXCXE peptide motif, while E1A utilizes its conserved region 1 (CR1) domain to interact with E2F and inhibits the p105-E2F interactions.

CDK PROTEIN INHIBITORS

CDK inhibitors are critical regulators of cell cycle progression and play essential roles in coordinating cell cycle arrest during processes such as the DDR (2). These inhibitors are broadly classified into two major families: the INK4 (Inhibitor of CDK4) family and the CIP/KIP (CDK-interacting protein/Kinase inhibitory protein) family, which differ in both their CDK specificity and their mode of interaction with CDK complexes (81). The INK4 family, which includes $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$, selectively inhibits CDK4 and CDK6 by binding monomeric CDK4/6, thereby preventing their association with D-type cyclins and ultimately blocking phosphorylation of the RB (82). In contrast, members of the CIP/KIP family, including $p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$, interact with and inhibit a broad spectrum of cyclin-CDK heterodimeric complexes, particularly cyclin E-CDK2 and cyclin A-CDK2 complexes, thereby restraining progression through the G1/S transition (22, 81).

Among these inhibitors, p21 was the first identified CDK inhibitor and represents a key transcriptional target of p53. By inhibiting cyclin E-CDK2 and cyclin A-CDK2 complexes, p21 prevents RB hyperphosphorylation and enforces cell cycle arrest, thereby contributing to tumor suppression (21, 83-85). Similarly, p18 functions as an inhibitor of CDK4/6, primarily during the G1 phase (2). Interestingly, there were attempts made to generate kinase inhibitors by altering cyclin-CDK complexes by resembling the functions of typical p21, p16, and p27, or via regulation of the cyclin-CDK targets (86).

A central regulatory node linking the RB and p53 tumor suppressor pathways is the *CDKN2A* locus, which encodes two distinct tumor suppressor proteins through alternative reading frames. $p16^{INK4A}$, a member of the INK4 family that regulates the RB pathway, and $p14^{ARF}$ in humans ($p19^{ARF}$ in mice), which functions in the p53 regulatory network (87, 88). $p16^{INK4A}$ induces G1 arrest by inhibiting CDK4/6 activity, thereby preventing RB phosphorylation and maintaining RB in its growth-suppressive state (19, 89). In contrast, ARF functions primarily through the p53-Murine Double Minute 2 (MDM2) pathway. Studies using mouse models demonstrated that murine p19ARF interacts with both p53 and the E3 ubiquitin ligase MDM2, highlighting the importance of ARF in regulating p53 stability. MDM2 is a major negative regulator of p53 as in fact it promotes p53 ubiquitination and proteasomal degradation (88). ARF antagonizes this process by binding to MDM2 and inhibiting its ligase activity, thereby stabilizing and activating p53. This regulatory axis forms a critical checkpoint in which ARF-mediated inhibition of MDM2 leads to p53 accumulation, resulting in transcriptional activation of p53 target genes involved in cell cycle arrest, apoptosis, and DDR (90).

Activation of p53 further reinforces cell cycle control by promoting transcription of p21, which suppresses CDK activity and reduces CDK-mediated phosphorylation of RB, thereby limiting the release of E2F transcription factors and inhibiting cell cycle progression (91). Post-translational modifications of p53, including phosphorylation at residues such as Ser15, Thr18, Ser20, Ser37 and Ser106, disrupt the p53-MDM2 interaction and contribute to p53 stabilization. In response to DNA damage, Ser15 is phosphorylated by ATM and can also be targeted by ATR (92, 93) while Ser37 is phosphorylated by ATR and DNA-PK (93, 94). Phosphorylation of Thr18 is mediated by casein kinase 1 (CK1) in Ser15-primed cascade (95). Ser20 is phosphorylated by the checkpoint kinase Chk2 (96), and Ser106 has been identified as an Aurora-A phosphorylation site that inhibits p53-MDM2 binding and prolongs p53 half-life (97). These phosphorylation events are often associated with acetylation events that further inhibit MDM2-mediated ubiquitination of p53, enhancing its tumor suppressor activity (88).

Importantly, alterations in endogenous cell cycle inhibitors are frequent events in human cancers. These alterations can occur through genetic deletion, transcriptional downregulation, or abnormal subcellular localization of CDK inhibitors. Notably, loss of the *CDKN2A* locus simultaneously disrupts both the RB pathway (via loss of $p16^{INK4A}$) and the p53 regulatory axis (via loss of ARF), representing a common oncogenic event that contributes to uncontrolled proliferation and tumor development.

CDK4/6 INHIBITORS

Although CDKs rapidly emerged as attractive therapeutic targets, the development of effective CDK inhibitors initially faced significant challenges (98). CDKs are highly conserved kinases across eukaryotic species, from yeast to humans, and their catalytic domains share strong homology, particularly within the ATP-binding pocket (15, 31). As a result, early generations of ATP-competitive CDK inhibitors lacked sufficient selectivity and inhibited multiple CDK family members (99). While these compounds showed promising activity in preclinical models, their clinical efficacy was limited because they targeted not only cell-cycle CDKs such as CDK4 and CDK6 but also transcriptional CDKs (*e.g.*, CDK7, CDK9) and kinases involved in DDR pathways (12). This broad inhibition often resulted in significant toxicity and limited therapeutic windows, highlighting the need for more selective strategies targeting specific CDK complexes in cancer.

In response to these limitations, alternative strategies for targeting CDKs have been explored. These include the development of highly selective ATP-competitive inhibitors specifically targeting CDK4/6, as well as non-ATP-competitive approaches designed to interfere with CDK-cyclin interactions, substrate recognition, or regulatory complex assembly (100). More recently, emerging technologies such as proteolysis-targeting chimeras (PROTACs) and molecular glues have been investigated to selectively degrade CDKs or disrupt CDK-associated regulatory networks (12). These approaches are aimed at improving spec-

ificity while minimizing off-target effects associated with earlier broad-spectrum CDK inhibitors.

Because dysregulation of CDK4/6 is frequently associated with cell cycle perturbance and cancer initiation, these kinases have always been attractive targets for therapeutic intervention but only recently they finally were successfully introduced in the clinical setting (101). CDK4/6 inhibitors work by blocking CDK4/6 activity (**Figure 2B**), thereby inhibiting RB phosphorylation, and preventing the transition from the G1 to S phase (102, 103). In clinical practice, CDK4/6 inhibitors have substantially improved the therapeutic options for $HR^+/HER2^-$ breast cancer (104). Several CDK inhibitors are in clinical trials, which can function as single agents and in combination with anticancer therapies (105). In this section, we will describe first-, second- and last generation CDK inhibitors and their main features.

Roscovitine is first-generation CDK inhibitor (106), and it is a broad-range purine inhibitor acting through direct competition at the conserved kinase ATP-binding site (34). Roscovitine inhibits CDK1, CDK2, CDK5, CDK7, and CDK9 but not CDK4 or CDK6 (107, 108). Also, this inhibitor primarily targets CDK2 (108). Roscovitine demonstrated therapeutic efficacy in diffuse pleural mesothelioma (DPM) cells when used in combination with AKT inhibitors (8, 109, 110) and enhanced the therapeutic efficacy of anti-PD1 in non-small cell lung cancer (109). This CDK inhibitor can also induce apoptosis through a p53-dependent pathway (111). An additional first-generation CDK inhibitor is flavopiridol (112), which blocks the activity of multiple CDKs, as in fact it is considered a pan-CDK inhibitor. Flavopiridol was developed after animal model studies demonstrated a tissue-specific necessity of single CDKs (113). However, flavopiridol is highly cytotoxic for patients, as in fact it is associated with gastrointestinal toxicity and extreme neutropenia, especially in metastatic breast cancer (112).

Palbociclib, ribociclib, and abemaciclib are defined as second-generation CDK inhibitors and were designed to specifically target CDK4/6 by binding to the ATP-binding pocket of CDK4/6, and block CDK4/6 activity (8, 103, 114). In contrast to the highly selective CDK4/6 inhibitors, dinaciclib is a broader-spectrum CDK inhibitor that targets CDK1, CDK2, CDK5, and CDK9, and has been evaluated in clinical trials for multiple malignancies rather than being approved for breast cancer therapy (115). Palbociclib was the first CDK4/6 inhibitor developed and was first approved by the FDA in 2015 for breast cancer therapy (4), followed by ribociclib and abemaciclib, which were approved by the FDA in 2017 (116). Accordingly, these three agents share the same core cytostatic mechanism CDK4/6 inhibition leading to blockade of RB phosphorylation and G1/S progression, whereas clinically relevant differences are primarily reflected in their safety profiles (10, 101).

The integration of palbociclib into the therapeutic regimen for $HR^+/HER2^-$ advanced breast cancer, in combination with endocrine therapy, has demonstrated a substantial increase in progression-free survival compared to endocrine monotherapy alone. Safety profile analysis highlights that grade 3-4 neutropenia represents the most significant adverse event, with a frequency of approximately 66% (117). However, the cytostatic nature and reversibility of this toxicity render febrile neutropenia a rare event, occurring in less than 2% of cases (117). In terms of relative comparison with other CDK4/6 inhibitors, palbociclib is characterized by superior gastrointestinal tolerability, a feature that clearly distinguishes it from the safety profile of abemaciclib (117).

Abemaciclib differs from other CDK4/6 inhibitors for its better selectivity for CDK4, a biochemical property that allows for continuous daily administration and results in a unique toxicity profile (118). In combination with endocrine therapy, the efficacy of abemaciclib has been extensively demonstrated in the MONARCH 3 trial, where it showed significant clinical benefit in both progression-free survival and overall survival (118). Unlike palbociclib and ribociclib, abemaciclib exhibits significantly lower rates of grade 3-4 neutropenia, at approximately 25-27% (117). However, the most characteristic adverse event is gastrointestinal toxicity; diarrhea reached grade 3 in 10-15% of cases, usually during the first treatment cycles (119). Other specific complications include an increased risk of venous thromboembolic events and an elevation in serum creatinine levels, the latter due to the inhibition of renal transporters and not indicative of actual organ damage (118).

Ribociclib, used in combination with endocrine therapy, has demonstrated robust clinical efficacy across various therapeutic settings, as confirmed by the results of the MONALEESA-2 and MONALEESA-3 clinical trials (120, 121). Similar to palbociclib, the safety profile of ribociclib is characterized by significant hematological toxicity, with grade 3-4 neutropenia being the most common adverse event, occurring in approximately 60% of patients (122). However, ribociclib is distinguished by specific non-hematological toxicities that require rigorous clinical mon-

itoring. In particular, it is associated with a risk of dose-dependent QTc interval prolongation, which occurs in about 3% of cases, necessitating periodic electrocardiographic monitoring (121). Furthermore, the drug shows a relevant incidence of hepatotoxicity, with grade 3-4 increases in transaminases (ALT/AST) reported in approximately 8-9% of patients, typically within the first six months of treatment (120). Dosage adjustments are frequently necessary to manage these specific toxicities; therefore, CDK4/6 inhibitors should be utilized in clinical practice to select the most appropriate drug, taking into account both efficacy and safety (104).

CDK4/6 inhibitors have demonstrated high efficacy as single agents and in combination therapies, particularly in HR^+ breast cancer. Multiple phase III clinical trials evaluating CDK4/6 inhibitors such as Palbociclib, Ribociclib, and Abemaciclib have consistently shown improved progression-free and overall survival when combined with endocrine therapy in $HR^+/HER2^-$ metastatic breast cancer (123). Importantly, these trials generally enroll patients whose tumors retain functional p105, as RB activity is required for CDK4/6 inhibitor-mediated cell-cycle arrest. In contrast, clinical efficacy in TNBC has been limited. TNBC frequently exhibits low p107 expression or p107 loss, which renders tumors intrinsically resistant to CDK4/6 inhibition because these drugs rely on RB-dependent suppression of E2F-driven transcription. Consequently, most clinical trials have focused on HR^+ disease rather than TNBC, and TNBC patients have largely been excluded or have shown minimal benefit. However, emerging evidence suggests that CDK4/6 inhibition may exert additional effects independently of their effect on the canonical RB-dependent pathway. For example, in RB-deficient TNBC, CDK4/6 inhibition can reduce mutant p53 protein stability through the RBM38 pathway, suggesting that mutant p53 status may serve as an additional biomarker of response and expanding the potential therapeutic scope of these agents (124). To overcome resistance and broaden their therapeutic applicability, CDK4/6 inhibitors are also actively explored in combination strategies. These include combinations with chemotherapeutic agents in breast cancer patients resistant to endocrine therapy (103), as well as with endocrine therapies, immunotherapy, and targeted therapies. Although early-phase studies have examined these strategies in TNBC, clinical responses remain modest and appear largely restricted to tumors retaining RB function.

Importantly, paclitaxel is a commonly used taxane that can be administered alone via IV infusion or in combination with additional anticancer drugs (125). Paclitaxel is used in combination with CDK4/6 inhibitors, especially with palbociclib (103). This dual targeting strategy effectively regulates the cell cycle and increases anticancer treatment efficacy. The combination therapy using paclitaxel and the CDK4/6 inhibitor palbociclib was highly effective and significantly minimized side effects associated with paclitaxel alone. A clinical study by Clark *et al*. (2019), on RB^+ advanced breast cancer patients treated with a paclitaxel-palbociclib combination therapy, showed improved patient outcomes without severe side effects (126). Patients first received intermittent doses of oral palbociclib and paclitaxel every other week. Patients who had a plateau response at more than 6 cycles in this clinical study design were able to continue their treatments with just palbociclib. Their dose increased one level higher than their previous combinatorial dose. Palbociclib and paclitaxel in RB^+ advanced breast cancer patients proved to be safe and effective without toxicity in RB^+ breast cancer.

CDK4/6 inhibitors, especially abemaciclib, can also be effectively used in combination with aromatase inhibitors to treat breast cancer (118). The aromatase inhibitors are classified into two groups: steroidal (type I) and nonsteroidal (type II). Anastrozole and letrozole are nonsteroidal aromatase inhibitors, which are highly effective in breast cancer therapy. For treatment of HER^+ and $HER2^-$ advanced breast cancer, abemaciclib was used in combination with these two aromatase inhibitors in the MONARCH-3 (randomized phase III) study, abemaciclib and anastrozole or letrozole greatly improved progression-free survival as well as an objective response rate. These treatments were safe for women with these advanced breast cancers (118).

Collectively, abemaciclib is considered highly effective for the treatment of HR^+ and HER^- breast cancer and exhibited significant efficacy when used as a second-line treatment in combination with fulvestrant, an anti-estrogen drug (8, 118). Sledge Jr *et al.* (2017) demonstrated that the abemaciclib-fulvestrant dual treatment was more effective than fulvestrant alone, as it was associated with increased cancer progression-free survival in patients with $HER^+/HER2^-$ advanced breast cancer (127). In advanced breast cancer, abemaciclib or palbociclib were effective in combination with fulvestrant (128). The PALOMA-3 phase III trial was conducted in a 2:1 randomized ratio, multicenter, double-blind, and placebo-con-

trolled to examine the safety and efficacy of a palbociclib-fulvestrant combined therapy (128). Patients treated with palbociclib-fulvestrant showed improvement in progression-free survival, while patients treated with placebo-fulvestrant did not significantly improve.

Similar to breast cancers, abemaciclib was effective in endometrial cancers (ECs) to prevent tumor progression (129). Treating ECs is very crucial as ECs are the sixth most common cancer in women, and the thirteenth most lethal globally (29, 130). ECs have dysregulated CDK4/6, but CDK4 plays a more prevalent role (129). Abemaciclib targets CDK4/6 with higher efficacy towards CDK4 inhibition, which is therefore highly beneficial for treating ECs. In addition, a combination therapy approach using the aromatase inhibitor letrozole and abemaciclib demonstrated encouraging efficacy in recurrent ER$^+$ endometrioid EC (131).

Costa *et al*. (2024) recently demonstrated that treating cisplatin-resistant DPM cell lines with abemaciclib was very effective in inhibiting growth and 3D spheroid formation of these cells. It was suggested that abemaciclib treatment may help in overcoming cisplatin-resistance in mesothelioma cells (8) (**Table 1**).

CDK4/6 inhibitor resistance is associated with abnormalities in the critical molecules of the cyclin D-CDK4/6-RB regulatory axis (114). FAT atypical cadherin 1 (FAT1) protocadherin tumor suppressor protein modulates CDK4/6 activity and may play a role in modulating resistance to CDK4/6 inhibitors (103), as in fact loss of function (LOF) mutations impact

Table 1. *Combination therapies involving CDK4/6 inhibitors.*

CDK4/6 INHIBITOR	COMBINATION PARTNER	CANCER TYPE	STUDY / TRIAL	KEY FINDINGS	REFERENCE
Palbociclib	Paclitaxel	RB$^+$ advanced breast cancer	Phase I/II clinical study	Combination improved patient outcomes and was well tolerated.	(126)
Abemaciclib	Aromatase inhibitors (anastrozole or letrozole)	HR$^+$/HER2$^-$ advanced breast cancer	MONARCH-3 (Phase III)	Significant improvement in progression-free survival and objective response rate compared to endocrine therapy alone	(118)
Abemaciclib	Fulvestrant	HR$^+$/HER2$^-$ advanced breast cancer	MONARCH-2	Combination significantly improved progression-free survival *versus* fulvestrant alone	(127)
Palbociclib	Fulvestrant	HR$^+$/HER2$^-$ advanced breast cancer	PALOMA-3 (Phase III)	Improved progression-free survival compared to placebo-fulvestrant	(128)
Palbociclib / Abemaciclib	Fulvestrant	Advanced breast cancer	Multiple clinical studies	Endocrine therapy plus CDK4/6 inhibition improved outcomes compared to endocrine therapy alone	(128)
Abemaciclib	Letrozole	ER$^+$ endometrioid endometrial cancer	Clinical evaluation	Combination showed encouraging efficacy in recurrent ER$^+$ endometrial cancer	(131)
Abemaciclib	Cisplatin-resistant tumor models	Mesothelioma	Preclinical study	Abemaciclib inhibited growth and 3D spheroid formation in cisplatin-resistant mesothelioma cells	(8)
Atirmociclib (CDK4-selective)	Endocrine therapy ± CDK2 inhibition / HER2 antibodies / immune checkpoint inhibitors	HR$^+$ breast cancer	Preclinical / translational studies	Selective CDK4 targeting improves anti-tumor efficacy and combination therapies may overcome resistance	(132)

Table 2. *Mechanisms of resistance to CDK4/6 inhibitors.*

RESISTANCE MECHANISM	MOLECULAR PATHWAY INVOLVED	BIOLOGICAL EFFECT	CLINICAL RELEVANCE	REFERENCE
FAT1 loss-of-function mutations	Hippo pathway activation	Increased CDK6 expression leading to resistance to CDK4/6 inhibitors	Observed in several cancers. Potential predictive biomarker	(103, 133)
Dysregulation of cyclin D-CDK4/6-RB axis	Cell cycle pathway	Reactivation of cell cycle progression despite CDK4/6 inhibition	Major driver of intrinsic and acquired resistance	(114)
Hippo pathway alterations (YAP, MST1, LATS1, NF2)	Hippo signaling	Increased proliferation and reduced sensitivity to CDK4/6 inhibition	Potential therapeutic targets	(103)
Wnt/β-catenin pathway activation	Wnt signaling	Promotes tumor progression and CDK4/6 inhibitor resistance	Linked to FAT1 mutations	(103)
MAPK/ERK pathway activation	MAPK signaling	Increased cell proliferation and survival	Contributes to therapeutic resistance	(103)

FAT1 and mediate CDK4/6 inhibitor resistance (132). FAT1 LOF activates the Hippo pathway, as it induces CDK6 expression, leading to resistance to CDK4/6 inhibitors (103, 132). FAT1 is often mutated in human cancers, therefore leading to tumor progression and an impact on cancer prognosis (103). Genomic analysis has demonstrated that FAT1 loss-of-function (LOF) mutations occur in approximately 5.8% of patients with metastatic breast cancer. The clinical relevance of this mechanism is underscored by a significant reduction in progression-free survival (PFS), which decreased from 10.1 months in FAT1 wild-type patients to 2.4 months in patients carrying the mutation, identifying *FAT1* loss as a robust predictive biomarker of poor response (132). FAT1 regulates the Wnt/β-catenin signaling pathway as well as the Hippo pathway and the MAPK/ERK pathways via protein-protein interactions, which affect cell proliferation, cell migration, and invasion (103). The YAP, MST1, LATS1, and NF2 proteins can also affect resistance to CDK4/6 inhibitors. While FAT1 targeting shows promise for reversing CDK4/6 inhibitor resistance, there is still a need for additional research to fully understand how FAT1-dependent regulatory mechanisms can cause resistance to CDK4/6 therapy. Despite the remarkable clinical success of CDK4/6 inhibitors in HR^+ breast cancer, both intrinsic and acquired resistance often limit long-term therapeutic benefits. Resistance mechanisms can arise through multiple molecular alterations that restore cell-cycle progression or activate compensatory proliferative pathways (114, 133). These mechanisms include loss or functional inactivation of *RB1*, amplification of *CCNE1* leading to increased CDK2 activity, upregulation of CDK6 expression, and activation of parallel signaling pathways such as PI3K/AKT/mTOR and MAPK signaling (103). Additional mechanisms involve alterations in upstream regulators of the CDK4/6-RB axis, epigenetic changes affecting cell cycle gene expression, and tumor microenvironment-mediated adaptive responses (101, 114, 134). Understanding these diverse resistance pathways is essential for designing rational combination strategies aimed at improving clinical efficacy of CDK4/6 inhibition (**Table 2**).

There are significant side effects associated with CDK4/6 inhibitors (1). Nonetheless, CDK4/6 inhibitors showed better efficacy than endocrine therapies (135). Abemaciclib is associated with the most significant adverse events, including diarrhea, anemia, neutropenia, nausea, and fatigue, especially when combined with endocrine therapies (10, 101). Abemaciclib can also trigger gastrointestinal-linked toxicities in comparison to palbociclib. Palbociclib can cause leukopenia, and fatigue, and ribociclib can lead to decreased leukocyte counts, neutropenia, elevated liver enzymes and prolonged QT levels. Ribociclib can also cause skin rash, fatigue, hypokalemia, and hyponatremia (107). Ribociclib is the CDK4/6 inhibitor with the highest risk of mortality, especially when used in combination with endocrine therapies. The use of ribociclib necessitates extreme caution, especially in patients with preexisting cardiovascular conditions. Patients put on ribociclib are therefore recommended to be closely monitored. These three inhibitors can all lead to mye-

Table 3. *Pharmacological properties of second-generation CDK4/6 inhibitors. They all have greater efficacy over CDK4 and CDK6, but can also inhibit CDK1 and CDK5.*

DRUG	PRIMARY TARGETS	ADDITIONAL TARGETS	PHARMACOKINETICS (HALF-LIFE, TMAX)
Palbociclib	Comparable activity against CDK4 and CDK6: • Cyclin D1-CDK4 • Cyclin D1/2/3-CDK6	• CDK1-Cyclin B • CDK5-p25	Half-life: ~26-27 h Tmax: 4-6 h
Ribociclib	Greater selectivity for CDK4 over CDK6: • Cyclin D1-CDK4 • Cyclin D1/2/3-CDK6	• CDK1-Cyclin B • CDK5 – -p25	Half-life: ~32-42 h Tmax: 1-5 h
Abemaciclib	Preferential inhibition of CDK4 over CDK6: • Cyclin D1-CDK4 • Cyclin D1/2/3-CDK6	• CDK1-Cyclin B • CDK5-p25	Half-life: ~17-38 h Tmax: 4-6 h
Atirmociclib (PF-07220060)	Highly selective for CDK4: • Cyclin D1-CDK4	• Cyclin E-CDK2	Terminal half-life: ~26.5 h Tmax: Not yet determined

losuppression and neutropenia (101). Collectively, these observations support the notion that palbociclib, ribociclib, and abemaciclib share a common on-target cytostatic mechanism, while drug-specific toxicity patterns likely reflect differences in target engagement and tissue dependence on CDK4 *versus* CDK6 (136).

Significantly, very recent work by Palmer *et al*. (2025) demonstrated that HR$^+$ breast cancer cells are significantly more dependent on CDK4 activity than CDK6, which is instead critical for hematopoiesis (136). Based on this observation, the researchers developed a next-generation selective CDK4 inhibitor, atirmociclib (PF-07220060), which showed enhanced anti-tumor efficacy and reduced toxicity compared to dual CDK4/6 inhibitors. Notably, the combination of endocrine therapy with either CDK2 inhibition, HER2 antibodies, or immune checkpoint inhibitors further increased atirmociclib efficacy, suggesting that dual therapy might counteract acquired resistance to CDK4 selective targeting (136). These results are very important as, in fact, they strongly support the expansion of more specific cell cycle-targeted therapy. A summary of current CDK4/6 inhibitor is listed in **Table 3**.

ANTI-CDK-RELATED CANCER THERAPIES

Dihydroartemisinin (DHA) is an immunotherapeutic anticancer drug that blocks cell proliferation and prevents tumor metastasis and angiogenesis in patients with colorectal cancer (CRC) (137). DHA also promotes immunogenic cell death (ICD) in hepatocellular carcinoma (HCC) (138). DHA therapies can even remodel the TME, boost cancer immunotherapy and enhance the efficacy of chimeric antigen receptor-T (CAR-T) cell-mediated tumor suppression and anti-programmed death-1 (anti-PD-1) antibody (**Figure 3**).

DHA leads to CDK inhibition (CDK1/2/4/5/6), as DHA upregulates intracellular reactive oxygen species (ROS), and an increase in ICD. ROS are critical factors associated with ICD since its immunogenicity weakens the N-Acetyl-L-cysteine antioxidant. The accumulation of ROS increases the amount of damage-associated molecular patterns (DAMPs) during the pre-apoptosis phase, thereby leading to the increase of ICD.

As DHA can inhibit CDKs, it can increase intracellular ROS and subsequently trigger apoptosis, cell cycle arrest, and autophagy (138). DHA can inhibit CDK4/6, but it mainly inhibits CDK4 (139). For example, DHA effectively inhibits cyclin D1-CDK4-RB signaling pathway in GC. DHA prevents GC cell proliferation and promotes cell cycle arrest. Fan *et al.*, discovered from flow cytometry analysis that DHA delivery increased the number of GC cells in G0/G1 transition phase, thereby lowering the number of cells in the S phase (139).

As demonstrated in HCC mouse model studies by Zhou *et al*. (2024), DHA treatment of HCC significantly increases the number of tumor-infiltrating CD8$^+$ T cells, which express CD25 and CD69 (138). Clinically, patients successfully treated with DHA

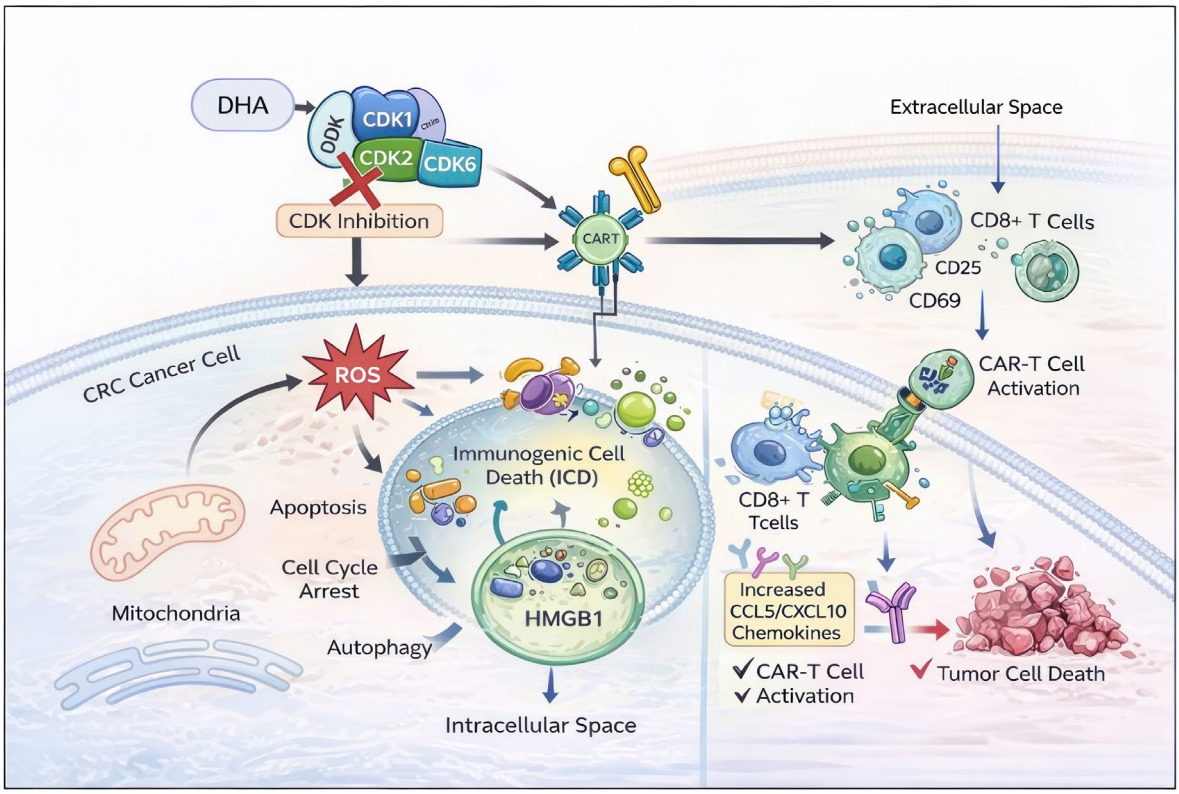

Figure 3. *DHA-mediated CDK inhibition promotes ROS-dependent immunogenic cell death and enhances antitumor immune responses. DHA inhibits multiple CDKs (CDK1, CDK2, CDK4, CDK5, and CDK6), with predominant inhibition of CDK4/6, leading to suppression of cyclin-dependent cell cycle progression in CRC cells. CDK inhibition by DHA results in increased intracellular ROS, which trigger apoptosis, cell cycle arrest, and autophagy. Elevated ROS levels promote ICD, characterized by the release of DAMPs, including HMGB1, during the pre-apoptotic phase. ICD subsequently enhances antitumor immunity by increasing tumor immunogenicity and stimulating immune cell infiltration. DHA treatment is associated with increased expression of chemokines CCL5 and CXCL10, facilitating recruitment and activation of CD8+ T cells expressing activation markers CD25 and CD69. In parallel, DHA remodels the TME and potentiates CAR-T–mediated antitumor responses, ultimately leading to enhanced tumor cell death.*

had increased mRNA levels of chemokines CCL5 and CXCL10. These clinical results indicate how DHA can inhibit dysregulated CDKs. The clinical prosperous results reaffirm the potential of ROS to suppress CDK-caused tumors. Also, CCL5 and CXCL10 decrease the amount of tumor-infiltrating $CD8^+$ T cells in colon cancer and melanoma (138).

In addition, results from experiments conducted in Huh7 cells (immortalized human hepatoma cells) treated with DHA downregulated CDK1/2/4/5/6 expression (138). The same results were obtained in Hepa1-6 (murine hepatoma – spontaneous liver tumor), H22 (mouse hepatocellular carcinoma), and HCCLM3 (human hepatocellular carcinoma) cell lines and were then further validated by qPCR and Western blotting analysis in HCC tumors (138). Notably, while conventional cytotoxic therapies act by damaging DNA and activating cell-cycle checkpoints, leading to efficient inhibition of CDKs, by contrast, DHA-associated CDK suppression works predominantly downstream of ROS induction and ICD/TME remodeling (140).

An additional important aspect currently under investigation is the optimal timing and sequential treatment of CDK4/6 inhibitor-based therapies relative to chemotherapy, radiotherapy, or other neo-adjuvant or adjuvant treatments. Because CDK4/6 inhibitors induce a cytostatic G1 cell-cycle arrest, their concurrent administration with cytotoxic agents targeting actively proliferating cells may reduce therapeutic efficacy (14, 141, 142). Consequently, several studies have explored sequential treatment strategies in which CDK4/6 inhibition is temporarily paused during chemotherapy or radiation exposure (143, 144). Conversely, in other contexts, CDK4/6 inhibitors may enhance antitumor immunity or promote tumor cell senescence, thereby potentiating the efficacy of immunotherapies or endocrine treatments (145, 146). Determining the optimal therapeutic scheduling of CDK4/6 inhibitors, therefore,

represents an important area of ongoing clinical investigation.

DISCUSSION AND CONCLUSION

CDKs are essential kinases for cell cycle progression, cell proliferation, differentiation, and DDR and act in concert with cyclins and RB proteins. CDK4/6 activity at the G1/S checkpoint is required for cell cycle progression, and dysregulation of CDK4/6 disrupts the cell cycle and promotes cancer initiation. CDK4/6 inhibitors have demonstrated significant efficacy in cancer therapies, as a monotherapy or in a combinational approach. Additionally, atirmociclib, a recently developed CDK4-specific inhibitor has shown great promises and enhanced safety as compared to classic CDK4/6 dual inhibitors. Nevertheless, some limitations should be acknowledged, including the limited availability of long-term clinical data and the rapidly expanding landscape of CDK-targeted therapies, which may lead to the emergence of additional therapeutic strategies beyond those discussed here. In conclusion, CDK inhibitors are emerging as important tools for cancer therapy. Future studies are aimed at better integrating CDK4/6 inhibitors with new immunotherapeutic therapies and/or personalized treatment modalities to further improve safety and clinical response.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

Funds from the Sbarro Health Research Organization supported this work.

Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Authors' contributions

SBA: writing – original draft, writing – review & editing. VS, GM: writing – review & editing, visualization. SS: writing – review & editing. AM: supervision, writing – review & editing. AG: funding acquisition, writing – review & editing.

Publication ethics

Plagiarism

This is a review article, and all original studies are cited as appropriate. Figures were generated with the help of AI and Biorender.

Data falsification and fabrication

The contents of the article are original, and any overlaps with other articles are by the authors themselves and appropriately cited.

REFERENCES

1. Abate AA, Pentimalli F, Esposito L, Giordano A. ATP-noncompetitive CDK inhibitors for cancer therapy: an overview. Expert Opin Investig Drugs. 2013;22(7):895-906. doi: 10.1517/13543784.2013.798641.
2. MacLachlan TK, Sang N, Giordano A. Cyclins, cyclin-dependent kinases and cdk inhibitors: implications in cell cycle control and cancer. Crit Rev Eukaryot Gene Expr. 1995;5(2):127-56. doi: 10.1615/critreveukargeneexpr.v5.i2.20.
3. Tanriverdi O, Yildiz A. The Role of Speedy/RINGO Protein in Breast Cancer as a Future Biomarker. Cancer Diagn Progn. 2024;4(3):209-213. doi: 10.21873/cdp.10310.
4. Huang Y, Liu W, Zhao C, Shi X, Zhao Q, Jia J, Wang A. Targeting cyclin-dependent kinases: From pocket specificity to drug selectivity. Eur J Med Chem. 2024;275:116547. doi: 10.1016/j.ejmech.2024.116547.
5. Alfano L, Iannuzzi CA, Barone D, Forte IM, Ragosta MC, Cuomo M, et al. CDK9-55 guides the anaphase-promoting complex/cyclosome (APC/C) in choosing the DNA repair pathway choice. Oncogene. 2024;43(17):1263-1273. doi: 10.1038/s41388-024-02982-w.
6. Bagella L, Sun A, Tonini T, Abbadessa G, Cottone G, Paggi MG, et al. A small molecule based on the pRb2/p130 spacer domain leads to inhibition of cdk2 activity, cell cycle arrest and tumor growth reduction in vivo. Oncogene. 2007;26(13):1829-39. doi: 10.1038/sj.onc.1209987.
7. Zhang M, Zhang L, Hei R, Li X, Cai H, Wu X, et al. CDK inhibitors in cancer therapy, an overview of recent development. Am J Cancer Res. 2021;11(5):1913-1935.
8. Costa A, Forte IM, Pentimalli F, Iannuzzi CA, Alfano L, Capone F, et al. Pharmacological inhibition of

CDK4/6 impairs diffuse pleural mesothelioma 3D spheroid growth and reduces viability of cisplatin-resistant cells. Front Oncol. 2024;14:1418951. doi: 10.3389/fonc.2024.1418951.
9. Brown VD, Phillips RA, Gallie BL. Cumulative effect of phosphorylation of pRB on regulation of E2F activity. Mol Cell Biol. 1999;19(5):3246-56. doi: 10.1128/MCB.19.5.3246.
10. Dubey R, Makhija R, Sharma A, Sahu A, Asati V. Unveiling the promise of pyrimidine-modified CDK inhibitors in cancer treatment. Bioorg Chem. 2024;149:107508. doi: 10.1016/j.bioorg.2024.107508.
11. Huang MF, Wang YX, Chou YT, Lee DF. Therapeutic Strategies for RB1-Deficient Cancers: Intersecting Gene Regulation and Targeted Therapy. Cancers (Basel). 2024;16(8):1558. doi: 10.3390/cancers16081558.
12. Marei HE, Bedair K, Hasan A, Al-Mansoori L, Gaiba A, Morrione A, et al. Targeting CDKs in cancer therapy: advances in PROTACs and molecular glues. NPJ Precis Oncol. 2025;9(1):204. doi: 10.1038/s41698-025-00931-8.
13. Mademtzoglou D, Relaix F. From cyclins to CDKIs: Cell cycle regulation of skeletal muscle stem cell quiescence and activation. Exp Cell Res. 2022;420(1):113275. doi: 10.1016/j.yexcr.2022.113275.
14. Goel S, DeCristo MJ, McAllister SS, Zhao JJ. CDK4/6 Inhibition in Cancer: Beyond Cell Cycle Arrest. Trends Cell Biol. 2018;28(11):911-925. doi: 10.1016/j.tcb.2018.07.002.
15. Łukasik P, Załuski M, Gutowska I. Cyclin-Dependent Kinases (CDK) and Their Role in Diseases Development-Review. Int J Mol Sci. 2021;22(6):2935. doi: 10.3390/ijms22062935.
16. Hydbring P, Malumbres M, Sicinski P. Non-canonical functions of cell cycle cyclins and cyclin-dependent kinases. Nat Rev Mol Cell Biol. 2016;17(5):280-92. doi: 10.1038/nrm.2016.27.
17. Ohtsubo M, Roberts JM. Cyclin-dependent regulation of G1 in mammalian fibroblasts. Science. 1993;259(5103):1908-12. doi: 10.1126/science.8384376.
18. Cornwell JA, Crncec A, Afifi MM, Tang K, Amin R, Cappell SD. Loss of CDK4/6 activity in S/G2 phase leads to cell cycle reversal. Nature. 2023;619(7969):363-370. doi: 10.1038/s41586-023-06274-3.
19. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: From Discovery to Therapy. Cancer Discov. 2016;6(4):353-67. doi: 10.1158/2159-8290.CD-15-0894.
20. Henley SA, Dick FA. The retinoblastoma family of proteins and their regulatory functions in the mammalian cell division cycle. Cell Div. 2012;7(1):10. doi: 10.1186/1747-1028-7-10.
21. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell. 1993;75(4):805-16. doi: 10.1016/0092-8674(93)90499-g.
22. Orend G, Hunter T, Ruoslahti E. Cytoplasmic displacement of cyclin E-cdk2 inhibitors p21Cip1 and p27Kip1 in anchorage-independent cells. Oncogene. 1998;16(20):2575-83. doi: 10.1038/sj.onc.1201791.
23. Suryadinata R, Sadowski M, Sarcevic B. Control of cell cycle progression by phosphorylation of cyclin-dependent kinase (CDK) substrates. Biosci Rep. 2010 Mar 17;30(4):243-55. doi: 10.1042/BSR20090171.
24. Roy R, Gampa SC, Garimella SV. Role of specific CDKs in regulating DNA damage repair responses and replication stress. Curr Opin Pharmacol. 2024;79:102485. doi: 10.1016/j.coph.2024.102485.
25. Song G, Liu J, Tang X, Zhong J, Zeng Y, Zhang X, et al. Cell cycle checkpoint revolution: targeted therapies in the fight against malignant tumors. Front Pharmacol. 2024;15:1459057. doi: 10.3389/fphar.2024.1459057.
26. Khan MGM, Wang Y. Cell Cycle-Related Clinical Applications. Methods Mol Biol. 2022;2579:35-46. doi: 10.1007/978-1-0716-2736-5_3.
27. Graña X, Claudio PP, De Luca A, Sang N, Giordano A. PISSLRE, a human novel CDC2-related protein kinase. Oncogene. 1994;9(7):2097-103.
28. Brambilla R, Draetta G. Molecular cloning of PISSLRE, a novel putative member of the cdk family of protein serine/threonine kinases. Oncogene. 1994;9(10):3037-41.
29. Mangone L, Marinelli F, Bisceglia I, Braghiroli MB, Mastrofilippo V, Fortunato M, et al. Incidence And Clinical Outcome Of Endometrial Cancer: A Population-Based Cancer-Registry Study. ARO. 2023;3(4):147-154. doi: 10.48286/aro.2023.77.
30. Modi V, Dunbrack RL Jr. Defining a new nomenclature for the structures of active and inactive kinases. Proc Natl Acad Sci U S A. 2019;116(14):6818-6827. doi: 10.1073/pnas.1814279116.
31. Malumbres M. Cyclin-dependent kinases. Genome Biol. 2014;15(6):122. doi: 10.1186/gb4184.
32. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. Nat Rev Drug Discov. 2009;8(7):547-66. doi: 10.1038/nrd2907.

33. Guen VJ, Gamble C, Lees JA, Colas P. The awakening of the CDK10/Cyclin M protein kinase. Oncotarget. 2017;8(30):50174-50186. doi: 10.18632/oncotarget.15024.
34. Darzynkiewicz Z, Gong J, Juan G, Ardelt B, Traganos F. Cytometry of cyclin proteins. Cytometry. 1996;25(1):1-13. doi: 10.1002/(SICI)1097-0320(19960901)25:1<1::AID-CYTO1>3.0.CO;2-N.
35. Tatum NJ, Endicott JA. Chatterboxes: the structural and functional diversity of cyclins. Semin Cell Dev Biol. 2020;107:4-20. doi: 10.1016/j.semcdb.2020.04.021.
36. Saleban M, Harris EL, Poulter JA. D-Type Cyclins in Development and Disease. Genes (Basel). 2023;14(7):1445. doi: 10.3390/genes14071445.
37. Dumitru AMG, Compton DA. Identifying Cyclin A/Cdk1 Substrates in Mitosis in Human Cells. Methods Mol Biol. 2022;2415:175-182. doi: 10.1007/978-1-0716-1904-9_13.
38. Ito M. Factors controlling cyclin B expression. Plant Mol Biol. 2000;43(5-6):677-90. doi: 10.1023/a:1006336005587.
39. Yam CH, Fung TK, Poon RY. Cyclin A in cell cycle control and cancer. Cell Mol Life Sci. 2002;59(8):1317-26. doi: 10.1007/s00018-002-8510-y.
40. Koff A, Giordano A, Desai D, Yamashita K, Harper JW, Elledge S, et al. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. Science. 1992;257(5077):1689-94. doi: 10.1126/science.1388288.
41. Chu C, Geng Y, Zhou Y, Sicinski P. Cyclin E in normal physiology and disease states. Trends Cell Biol. 2021;31(9):732-746. doi: 10.1016/j.tcb.2021.05.001.
42. Bazzi ZA, Tai IT. CDK10 in Gastrointestinal Cancers: Dual Roles as a Tumor Suppressor and Oncogene. Front Oncol. 2021;11:655479. doi: 10.3389/fonc.2021.655479.
43. Swanton C, Downward J. Unraveling the complexity of endocrine resistance in breast cancer by functional genomics. Cancer Cell. 2008;13(2):83-5. doi: 10.1016/j.ccr.2008.01.021.
44. Iorns E, Turner NC, Elliott R, Syed N, Garrone O, Gasco M, et al. Identification of CDK10 as an important determinant of resistance to endocrine therapy for breast cancer. Cancer Cell. 2008;13(2):91-104. doi: 10.1016/j.ccr.2008.01.001.
45. Guen VJ, Gamble C, Flajolet M, Unger S, Thollet A, Ferandin Y, et al. CDK10/cyclin M is a protein kinase that controls ETS2 degradation and is deficient in STAR syndrome. Proc Natl Acad Sci U S A. 2013;110(48):19525-30. doi: 10.1073/pnas.1306814110.
46. Zhou X, Lu D, Yi W, Xu D. Downregulation of CDC25C in NPCs Disturbed Cortical Neurogenesis. Int J Mol Sci. 2023;24(2):1505. doi: 10.3390/ijms24021505.
47. Dakilah I, Harb A, Abu-Gharbieh E, El-Huneidi W, Taneera J, Hamoudi R, et al. Potential of CDC25 phosphatases in cancer research and treatment: key to precision medicine. Front Pharmacol. 2024;15:1324001. doi: 10.3389/fphar.2024.1324001.
48. Hoffmann I, Draetta G, Karsenti E. Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J. 1994;13(18):4302-10. doi: 10.1002/j.1460-2075.1994.tb06750.x.
49. Bona AB, Calcagno DQ, Ribeiro HF, Muniz JAPC, Pinto GR, Rocha CAM, Lacreta Junior ACC, de Assumpção PP, Herranz JAR, Burbano RR. Menadione reduces CDC25B expression and promotes tumor shrinkage in gastric cancer. Ther Adv Gastroenterol. 2020;13:1756284819895435. doi: 10.1177/1756284819895435.
50. Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. Curr Opin Cell Biol. 2006;18(2):185-91. doi: 10.1016/j.ceb.2006.02.003.
51. Flores M, Goodrich DW. Retinoblastoma Protein Paralogs and Tumor Suppression. Front Genet. 2022;13:818719. doi: 10.3389/fgene.2022.818719.
52. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature. 1986;323(6089):643-6. doi: 10.1038/323643a0.
53. Fung YK, Murphree AL, T'Ang A, Qian J, Hinrichs SH, Benedict WF. Structural evidence for the authenticity of the human retinoblastoma gene. Science. 1987;236(4809):1657-61. doi: 10.1126/science.2885916.
54. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science. 1987;235(4794):1394-9. doi: 10.1126/science.3823889.
55. Ma M, Zhu Y, Xiao C, Li R, Cao X, Kang R, et al. Novel insights into RB1 in prostate cancer lineage plasticity and drug resistance. Tumori. 2024;110(4):252-263. doi: 10.1177/03008916231225576.
56. Ventura E, Iannuzzi CA, Pentimalli F, Giordano A, Morrione A. RBL1/p107 Expression Levels Are

Modulated by Multiple Signaling Pathways. Cancers (Basel). 2021;13(19):5025. doi: 10.3390/cancers13195025.
57. Cao L, Peng B, Yao L, Zhang X, Sun K, Yang X, et al. The ancient function of RB-E2F pathway: insights from its evolutionary history. Biol Direct. 2010;5:55. doi: 10.1186/1745-6150-5-55.
58. Engeland K. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. Cell Death Differ. 2018;25(1):114-132. doi: 10.1038/cdd.2017.172.
59. Pentimalli F. Updates from the TP53 universe. Cell Death Differ. 2018;25(1):10-12. doi: 10.1038/cdd.2017.190.
60. Indovina P, Marcelli E, Casini N, Rizzo V, Giordano A. Emerging roles of RB family: new defense mechanisms against tumor progression. J Cell Physiol. 2013;228(3):525-35. doi: 10.1002/jcp.24170.
61. Indovina P, Pentimalli F, Casini N, Vocca I, Giordano A. RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. Oncotarget. 2015;6(20):17873-90. doi: 10.18632/oncotarget.4286.
62. Classon M, Dyson N. p107 and p130: versatile proteins with interesting pockets. Exp Cell Res. 2001;264(1):135-47. doi: 10.1006/excr.2000.5135.
63. Gaubatz S, Lindeman GJ, Ishida S, Jakoi L, Nevins JR, Livingston DM, et al. E2F4 and E2F5 play an essential role in pocket protein-mediated G1 control. Mol Cell. 2000;6(3):729-35. doi: 10.1016/s1097-2765(00)00071-x.
64. Liban TJ, Thwaites MJ, Dick FA, Rubin SM. Structural Conservation and E2F Binding Specificity within the Retinoblastoma Pocket Protein Family. J Mol Biol. 2016;428(20):3960-3971. doi: 10.1016/j.jmb.2016.08.017.
65. Hurford RK Jr, Cobrinik D, Lee MH, Dyson N. pRB and p107/p130 are required for the regulated expression of different sets of E2F responsive genes. Genes Dev. 1997;11(11):1447-63. doi: 10.1101/gad.11.11.1447.
66. Classon M, Salama S, Gorka C, Mulloy R, Braun P, Harlow E. Combinatorial roles for pRB, p107, and p130 in E2F-mediated cell cycle control. Proc Natl Acad Sci U S A. 2000;97(20):10820-5. doi: 10.1073/pnas.190343497.
67. Dick FA, Sailhamer E, Dyson NJ. Mutagenesis of the pRB pocket reveals that cell cycle arrest functions are separable from binding to viral oncoproteins. Mol Cell Biol. 2000;20(10):3715-27. doi: 10.1128/MCB.20.10.3715-3727.2000.
68. Mäkelä JA, Toppari J. Retinoblastoma-E2F Transcription Factor Interplay Is Essential for Testicular Development and Male Fertility. Front Endocrinol (Lausanne). 2022;13:903684. doi: 10.3389/fendo.2022.903684.
69. Swaidan R, Daher A, Bejjani AT, El Atie YE, Chehab Y, Bou Hamdan R, et al. Rb substantially compensates for the double loss of p130 and p107 in adult but not embryonic neural stem cell lineages. Cell Death Dis. 2025;16(1):511. doi: 10.1038/s41419-025-07815-6.
70. Stengel KR, Thangavel C, Solomon DA, Angus SP, Zheng Y, Knudsen ES. Retinoblastoma/p107/p130 pocket proteins: protein dynamics and interactions with target gene promoters. J Biol Chem. 2009;284(29):19265-71. doi: 10.1074/jbc.M808740200.
71. Sadasivam S, DeCaprio JA. The DREAM complex: master coordinator of cell cycle-dependent gene expression. Nat Rev Cancer. 2013;13(8):585-95. doi: 10.1038/nrc3556.
72. Bujarrabal-Dueso A, Sendtner G, Meyer DH, Chatzinikolaou G, Stratigi K, Garinis GA, et al. The DREAM complex functions as conserved master regulator of somatic DNA-repair capacities. Nat Struct Mol Biol. 2023;30(4):475-488. doi: 10.1038/s41594-023-00942-8.
73. Uxa S, Bernhart SH, Mages CFS, Fischer M, Kohler R, Hoffmann S, et al. DREAM and RB cooperate to induce gene repression and cell-cycle arrest in response to p53 activation. Nucleic Acids Res. 2019;47(17):9087-9103. doi: 10.1093/nar/gkz635.
74. Engeland K. Cell cycle regulation: p53-p21-RB signaling. Cell Death Differ. 2022;29(5):946-960. doi: 10.1038/s41418-022-00988-z.
75. Quaas M, Kohler R, Nöltner L, Schmidbauer LF, Uxa S, Müller GA, et al. BRCA1 and BRCA2 gene expression: p53- and cell cycle-dependent repression requires RB and DREAM. Cell Death Differ. 2026;33(1):51-63. doi: 10.1038/s41418-025-01566-9.
76. Mages CF, Wintsche A, Bernhart SH, Müller GA. The DREAM complex through its subunit Lin37 cooperates with Rb to initiate quiescence. Elife. 2017;6:e26876. doi: 10.7554/eLife.26876.
77. Pattschull G, Walz S, Gründl M, Schwab M, Rühl E, Baluapuri A, et al. The Myb-MuvB Complex Is Required for YAP-Dependent Transcription of Mitotic Genes. Cell Rep. 2019;27(12):3533-3546.e7. doi: 10.1016/j.celrep.2019.05.071.
78. Koliopoulos MG, Alfieri C. Cell cycle regulation by complex nanomachines. FEBS J. 2022;289(17):5100-5120. doi: 10.1111/febs.16082.

79. Whyte P. The retinoblastoma protein and its relatives. Semin Cancer Biol. 1995;6(2):83-90. doi: 10.1006/scbi.1995.0011.
80. Talluri S, Dick FA. Regulation of transcription and chromatin structure by pRB: here, there and everywhere. Cell Cycle. 2012;11(17):3189-98. doi: 10.4161/cc.21263.
81. Csergeová L, Krbušek D, Janoštiak R. CIP/KIP and INK4 families as hostages of oncogenic signaling. Cell Div. 2024;19(1):11. doi: 10.1186/s13008-024-00115-z.
82. Roussel MF. The INK4 family of cell cycle inhibitors in cancer. Oncogene. 1999;18(38):5311-7. doi: 10.1038/sj.onc.1202998.
83. Fischer M, Quaas M, Steiner L, Engeland K. The p53-p21-DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. Nucleic Acids Res. 2016;44(1):164-74. doi: 10.1093/nar/gkv927.
84. Kreis NN, Louwen F, Yuan J. The Multifaceted p21 (Cip1/Waf1/CDKN1A) in Cell Differentiation, Migration and Cancer Therapy. Cancers (Basel). 2019;11(9):1220. doi: 10.3390/cancers11091220.
85. Shamloo B, Usluer S. p21 in Cancer Research. Cancers (Basel). 2019;11(8):1178. doi: 10.3390/cancers11081178.
86. Cirillo D, Pentimalli F, Giordano A. Peptides or small molecules? Different approaches to develop more effective CDK inhibitors. Curr Med Chem. 2011;18(19):2854-66. doi: 10.2174/092986711796150496.
87. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc Natl Acad Sci U S A. 1998;95(14):8292-7. doi: 10.1073/pnas.95.14.8292.
88. Kung CP, Weber JD. It's Getting Complicated-A Fresh Look at p53-MDM2-ARF Triangle in Tumorigenesis and Cancer Therapy. Front Cell Dev Biol. 2022;10:818744. doi: 10.3389/fcell.2022.818744.
89. Ohtani N, Yamakoshi K, Takahashi A, Hara E. The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. J Med Invest. 2004;51(3-4):146-53. doi: 10.2152/jmi.51.146.
90. Nag S, Qin J, Srivenugopal KS, Wang M, Zhang R. The MDM2-p53 pathway revisited. J Biomed Res. 2013;27(4):254-71. doi: 10.7555/JBR.27.20130030.
91. Kuganesan N, Dlamini S, Tillekeratne LMV, Taylor WR. Tumor suppressor p53 promotes ferroptosis in oxidative stress conditions independent of modulation of ferroptosis by p21, CDKs, RB, and E2F. J Biol Chem. 2021;297(6):101365. doi: 10.1016/j.jbc.2021.101365.
92. Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science. 1998;281(5383):1677-9. doi: 10.1126/science.281.5383.1677.
93. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh SY, et al. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 1999;13(2):152-7. doi: 10.1101/gad.13.2.152.
94. Lees-Miller SP, Sakaguchi K, Ullrich SJ, Appella E, Anderson CW. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. Mol Cell Biol. 1992;12(11):5041-9. doi: 10.1128/mcb.12.11.5041-5049.1992.
95. Sakaguchi K, Saito S, Higashimoto Y, Roy S, Anderson CW, Appella E. Damage-mediated phosphorylation of human p53 threonine 18 through a cascade mediated by a casein 1-like kinase. Effect on Mdm2 binding. J Biol Chem. 2000;275(13):9278-83. doi: 10.1074/jbc.275.13.9278.
96. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000;287(5459):1824-7. doi: 10.1126/science.287.5459.1824.
97. Hsueh KW, Fu SL, Chang CB, Chang YL, Lin CH. A novel Aurora-A-mediated phosphorylation of p53 inhibits its interaction with MDM2. Biochim Biophys Acta. 2013;1834(2):508-15. doi: 10.1016/j.bbapap.2012.11.005.
98. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov. 2015;14(2):130-46. doi: 10.1038/nrd4504.
99. Rowland RJ, Noble MEM, Endicott JA. Diversity in CDK structural mechanisms of regulation and drug discovery opportunities. Trends Biochem Sci. 2026;51(3):234-248. doi: 10.1016/j.tibs.2025.12.007.
100. Hope I, Endicott JA, Watt JE. Emerging approaches to CDK inhibitor development, a structural perspective. RSC Chem Biol. 2022;4(2):146-164. doi: 10.1039/d2cb00201a.
101. Glaviano A, Wander SA, Baird RD, Yap KC, Lam HY, Toi M, et al. Mechanisms of sensitivity and resistance to CDK4/CDK6 inhibitors in hormone receptor-positive breast cancer treatment. Drug Resist Updat. 2024;76:101103. doi: 10.1016/j.drup.2024.101103.
102. Bolzacchini E, Pomero F, Fazio M, Civitelli C, Fabro G, Pellegrino D, et al. Risk of venous and

arterial thromboembolic events in women with advanced breast cancer treated with CDK 4/6 inhibitors: A systematic review and meta-analysis. Thromb Res. 2021;208:190-197. doi: 10.1016/j.thromres.2021.11.009.
103. Xue Y, Zhai J. Strategy of combining CDK4/6 inhibitors with other therapies and mechanisms of resistance. Int J Clin Exp Pathol. 2024;17(7):189-207. doi: 10.62347/HGNI4903.
104. Yang P, Zhang H, Wu M, Zhao F, Wang M, Zhao J, et al. Dose reduction and discontinuation due to the combination of CDK4/6 inhibitors and endocrine drugs: a systematic review and meta-analysis. Eur J Clin Pharmacol. 2024;80(12):1893-1902. doi: 10.1007/s00228-024-03757-8.
105. Esposito L, Indovina P, Magnotti F, Conti D, Giordano A. Anticancer therapeutic strategies based on CDK inhibitors. Curr Pharm Des. 2013;19(30):5327-32. doi: 10.2174/1381612811319990377.
106. Schang LM. Effects of pharmacological cyclin-dependent kinase inhibitors on viral transcription and replication. Biochim Biophys Acta. 2004;1697(1-2):197-209. doi: 10.1016/j.bbapap.2003.11.024.
107. Cicenas J, Kalyan K, Sorokinas A, Stankunas E, Levy J, Meskinyte I, et al. Roscovitine in cancer and other diseases. Ann Transl Med. 2015;3(10):135. doi: 10.3978/j.issn.2305-5839.2015.03.61.
108. Federico M, Symonds CE, Bagella L, Rizzolio F, Fanale D, Russo A, et al. R-Roscovitine (Seliciclib) prevents DNA damage-induced cyclin A1 upregulation and hinders non-homologous end-joining (NHEJ) DNA repair. Mol Cancer. 2010;9:208. doi: 10.1186/1476-4598-9-208.
109. Diaz-Montero CM, Holvey-Bates EG, Rayman PA, Parker Y, Lindner DJ, Stark GR, et al. The CDK inhibitor Roscovitine enhances the therapeutic efficacy of anti-PD-1 in non-small cell lung cancer. Front Oncol. 2026;15:1745967. doi: 10.3389/fonc.2025.1745967.
110. Bonelli MA, Digiacomo G, Fumarola C, Alfieri R, Quaini F, Falco A, et al. Combined Inhibition of CDK4/6 and PI3K/AKT/mTOR Pathways Induces a Synergistic Anti-Tumor Effect in Malignant Pleural Mesothelioma Cells. Neoplasia. 2017;19(8):637-648. doi: 10.1016/j.neo.2017.05.003.
111. Benot-Dominguez R, Cimini A, Barone D, Giordano A, Pentimalli F. The Emerging Role of Cyclin-Dependent Kinase Inhibitors in Treating Diet-Induced Obesity: New Opportunities for Breast and Ovarian Cancers? Cancers (Basel). 2022;14(11):2709. doi: 10.3390/cancers14112709.
112. Finn RS, Aleshin A, Slamon DJ. Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. Breast Cancer Res. 2016;18(1):17. doi: 10.1186/s13058-015-0661-5.
113. Rizzolio F, Tuccinardi T, Caligiuri I, Lucchetti C, Giordano A. CDK inhibitors: from the bench to clinical trials. Curr Drug Targets. 2010;11(3):279-90. doi: 10.2174/138945010790711978.
114. Huang J, Zheng L, Sun Z, Li J. CDK4/6 inhibitor resistance mechanisms and treatment strategies (Review). Int J Mol Med. 2022;50(4):128. doi: 10.3892/ijmm.2022.5184.
115. Rajput S, Khera N, Guo Z, Hoog J, Li S, Ma CX. Inhibition of cyclin dependent kinase 9 by dinaciclib suppresses cyclin B1 expression and tumor growth in triple negative breast cancer. Oncotarget. 2016;7(35):56864-56875. doi: 10.18632/oncotarget.10870.
116. Byers KF. Ribociclib and Abemaciclib: CDK4/6 Inhibitors for the Treatment of Hormone Receptor-Positive Metastatic Breast Cancer. J Adv Pract Oncol. 2021;12(1):100-107. doi: 10.6004/jadpro.2021.12.1.8.
117. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 2016;375(20):1925-1936. doi: 10.1056/NEJMoa1607303.
118. Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol. 2017;35(32):3638-3646. doi: 10.1200/JCO.2017.75.6155.
119. Sledge GW Jr, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. The Effect of Abemaciclib Plus Fulvestrant on Overall Survival in Hormone Receptor-Positive, ERBB2-Negative Breast Cancer That Progressed on Endocrine Therapy-MONARCH 2: A Randomized Clinical Trial. JAMA Oncol. 2020;6(1):116-124. doi: 10.1001/jamaoncol.2019.4782.
120. Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im SA, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol. 2018;36(24):2465-2472. doi: 10.1200/JCO.2018.78.9909.

121. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. N Engl J Med. 2016;375(18):1738-1748. doi: 10.1056/NEJMoa1609709.
122. Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. Lancet. 2020;395(10226):817-827. doi: 10.1016/S0140-6736(20)30165-3.
123. Buonaiuto R, Fordellone M, Caltavituro A, Cataldo ML, Criscitiello C, Dieci MV, et al. Efficacy and safety of systemic therapies following progression on CDK4/6 inhibitors in patients with $HR^+/HER2^-$ metastatic breast cancer: a systematic review and network meta-analysis. EClinicalMedicine. 2025;89:103535. doi: 10.1016/j.eclinm.2025.103535.
124. Zhang J, Wen K, Nakajima KI, Shi Y, Chen X. CDK4/6 Inhibitors Suppress RB-Null Triple-Negative Breast Cancer by Inhibiting Mutant P53 Expression via RBM38 RNA-Binding Protein. Cancers (Basel). 2025;17(20):3339. doi: 10.3390/cancers17203339.
125. Burk S, Morrione A. A brief overview of several recent advancements of targeted-therapies and antibody-conjugate drugs for advanced triple-negative breast cancer. ARO. 2023;3(2):86-98. doi: 10.48286/aro.2023.65.
126. Clark AS, McAndrew NP, Troxel A, Feldman M, Lal P, Rosen M, et al. Combination Paclitaxel and Palbociclib: Results of a Phase I Trial in Advanced Breast Cancer. Clin Cancer Res. 2019;25(7):2072-2079. doi: 10.1158/1078-0432.CCR-18-0790.
127. Sledge GW Jr, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With $HR^+/HER2^-$ Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. J Clin Oncol. 2017;35(25):2875-2884. doi: 10.1200/JCO.2017.73.7585.
128. Sammons S, Kornblum NS, Blackwell KL. Fulvestrant-Based Combination Therapy for Second-Line Treatment of Hormone Receptor-Positive Advanced Breast Cancer. Target Oncol. 2019;14(1):1-12. doi: 10.1007/s11523-018-0587-9.
129. Awada A, Ahmad S. Therapeutic Prospects of Abemaciclib for Patients with Endometrial Cancer. Curr Oncol. 2024;31(9):5374-5383. doi: 10.3390/curroncol31090397.
130. Martinelli C, Vidali A, Di Chiara F, Mazzarotti G, El Messaoudi S, Alfano L, Ercoli A, Giordano A. Endometriosis: A Cancer-Mimicking Disease And The Need For A Translational Perspective. ARO. 2025;5(2):75-94. doi: 10.48286/aro.2025.107.
131. Konstantinopoulos PA, Cai M, Lee EK, Krasner C, Campos SM, Liu JF, et al. Serial Circulating Tumor DNA Sequencing to Monitor Response and Define Acquired Resistance to Letrozole/Abemaciclib in Endometrial Cancer. JCO Precis Oncol. 2025;9:e2400882. doi: 10.1200/PO-24-00882.
132. Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, et al. Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. Cancer Cell. 2018;34(6):893-905.e8. doi: 10.1016/j.ccell.2018.11.006.
133. von Manstein V, Yang CM, Richter D, Delis N, Vafaizadeh V, Groner B. Resistance of Cancer Cells to Targeted Therapies Through the Activation of Compensating Signaling Loops. Curr Signal Transduct Ther. 2013;8(3):193-202. doi: 10.2174/15743624096661402062219 31.
134. Sawant M, Wilson A, Sridaran D, Mahajan K, O'Conor CJ, Hagemann IS, et al. Epigenetic reprogramming of cell cycle genes by ACK1 promotes breast cancer resistance to CDK4/6 inhibitor. Oncogene. 2023;42(29):2263-2277. doi: 10.1038/s41388-023-02747-x.
135. Petroni G, Formenti SC, Chen-Kiang S, Galluzzi L. Immunomodulation by anticancer cell cycle inhibitors. Nat Rev Immunol. 2020;20(11):669-679. doi: 10.1038/s41577-020-0300-y.
136. Palmer CL, Boras B, Pascual B, Li N, Li D, Garza S, et al. CDK4 selective inhibition improves preclinical anti-tumor efficacy and safety. Cancer Cell. 2025;43(3):464-481.e14. doi: 10.1016/j.ccell.2025.02.006.
137. Dai X, Zhang X, Chen W, Chen Y, Zhang Q, Mo S, et al. Dihydroartemisinin: A Potential Natural Anticancer Drug. Int J Biol Sci. 2021;17(2):603-622. doi: 10.7150/ijbs.50364.
138. Zhou Z, Lei J, Fang J, Chen P, Zhou J, Wang H, et al. Dihydroartemisinin remodels tumor micro-environment and improves cancer immunotherapy through inhibiting cyclin-dependent kinases. Int Immunopharmacol. 2024;139:112637. doi: 10.1016/j.intimp.2024.112637.
139. Fan HN, Zhu MY, Peng SQ, Zhu JS, Zhang J, Qu GQ. Dihydroartemisinin inhibits the growth and invasion of gastric cancer cells by regulating cyclin D1-CDK4-Rb signaling. Pathol

Res Pract. 2020;216(2):152795. doi: 10.1016/j.prp.2019.152795.
140. Benada J, Macurek L. Targeting the Checkpoint to Kill Cancer Cells. Biomolecules. 2015;5(3):1912-37. doi: 10.3390/biom5031912.
141. Jin D, Tran N, Thomas N, Tran DD. Combining CDK4/6 inhibitors ribociclib and palbociclib with cytotoxic agents does not enhance cytotoxicity. PLoS One. 2019;14(10):e0223555. doi: 10.1371/journal.pone.0223555.
142. Liu Y, Park S, Li Y. Breaking Cancer's Momentum: CDK4/6 Inhibitors and the Promise of Combination Therapy. Cancers (Basel). 2025;17(12):1941. doi: 10.3390/cancers17121941.
143. Brion T, Quéro L. Radiotherapy and CDK inhibitors: Opportunities and risks. Cancer Radiother. 2022;26(6-7):968-972. doi: 10.1016/j.canrad.2022.06.017.
144. Roberts PJ, Kumarasamy V, Witkiewicz AK, Knudsen ES. Chemotherapy and CDK4/6 Inhibitors: Unexpected Bedfellows. Mol Cancer Ther. 2020;19(8):1575-1588. doi: 10.1158/1535-7163.MCT-18-1161.
145. Scordamaglia D, Talia M, Zicarelli A, Mondino AA, De Rosis S, Di Dio M, et al. Alternate actions of CDK4/6 inhibitors beyond cell cycle blockade: unexplored roles in therapy resistance. Cancer Metastasis Rev. 2025;44(4):90. doi: 10.1007/s10555-025-10307-w.
146. Si Y, Li H, Shi Y. The Dual Effects of CDK4/6 Inhibitors on Tumor Immunity. Cancers (Basel). 2025;17(24):3997. doi: 10.3390/cancers17243997.