The regulatory role of CDK4/6 inhibitors in tumor immunity and the potential value of tumor immunotherapy (Review)

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Abstract. Cyclin-dependent kinase (CDK)4/6 inhibitors regulate the cell cycle by binding to CDK4/6, thus exerting an inhibitory effect, and they have a notable impact on tumor immunity. CDK4/6 inhibitors have been demonstrated to modulate the immune microenvironment by affecting immune cells and immune escape phenomena in the tumor microenvironment. T cells, natural killer cells and macrophages are all regulated by CDK4/6 inhibitors, thereby acting on cancer cells. In addition, these inhibitors modulate immune checkpoints, enhancing antitumor immune responses when combined with immune checkpoint inhibitors, such as programmed death-ligand 1 and programmed death-1. However, these inhibitors are not without limitations, as they can enhance tumor immune evasion. Therefore, combination therapies to improve efficacy are being investigated, including immunotherapy, targeted therapy, chemotherapy and radiation therapy. In addition, challenges associated with the widespread use of CDK4/6 inhibitors, such as the emergence of tumor resistance, underscore the necessity for further research to enhance the clinical applicability of these inhibitors.

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Contents

1. Introduction

Cyclin-dependent kinase (CDK)4/6 plays a key role in cell cycle regulation, where aberrant activation of the cyclin D-CDK4/6-retinoblastoma (Rb) gene axis often leads to unrestrained cell proliferation, one of the central mechanisms of tumorigenesis (1). Notably, CDK4/6 inhibitors, such as palbociclib, ribociclib and abemaciclib, have shown marked efficacy in the treatment of patients with hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer ($HR^+/HER2^-$ MBC) (2). Traditionally, CDK4/6 inhibitors inhibit cell proliferation by inhibiting Rb phosphorylation and inducing pre-DNA synthesis (G_1) cell cycle arrest in tumor cells (3). However, it has also been shown that CDK4/6 inhibitors are essential in modulating tumor immunity. For example, CDK4/6 inhibitors can promote tumor cytotoxic T-cell-mediated clearance by affecting tumor cells and can reduce the activity of E2F-targeted DNA methyltransferase 1 (DNMT1) in regulatory T cells (Tregs). In addition, CDK4/6 inhibitors can enhance the therapeutic effects of immune checkpoint inhibitors (ICIs) by upregulating antigen presentation mechanisms and programmed death-ligand 1 (PD-L1) expression, thus exhibiting good synergistic potential and providing new perspectives for tumor immunotherapy (4).

Despite their potential, CDK4/6 inhibitors still face challenges in the clinic. Clinical and preclinical studies have shown that ~20% of patients with breast cancer (BC) have innate resistance to CDK4/6 inhibitors (5), and >30% of

patients gradually develop acquired resistance during ongoing treatment (6), severely limiting the durability of their efficacy and widespread use. The mechanisms of resistance, whether innate or acquired, involve both compensatory activation of cell cycle-related pathways and multiple non-cell cycle escape strategies (7). In addition, the hematological toxicity of CDK4/6 inhibitors should not be ignored. In the PALOMA-3 study, 55.3% of patients developed grade 3 neutropenia and 9.7% developed grade 4 neutropenia, suggesting the importance of regular patient monitoring and dose adjustment to ensure safety (8). Therefore, future studies should focus on overcoming resistance to CDK4/6 inhibitors, searching for predictive biomarkers of therapeutic sensitivity, optimizing dosing regimens, and improving patient monitoring and dose adjustment to improve therapeutic efficacy and safety.

Based on the aforementioned information, the present study aims to systematically review the mechanism of CDK4/6 inhibitors in cell cycle regulation and tumor immunomodulation, and to thoroughly elaborate their drug resistance mechanisms, including the compensatory activation of cell cycle-related pathways and multiple non-cell cycle escape strategies. The review also discusses the recent progress and future directions in overcoming drug resistance and optimizing clinical application, with the aim of providing a theoretical basis and practical guidance for the future use of precision therapy with CDK4/6 inhibitors.

2. Fundamental mechanisms of action and cell cycle regulation of CDK4/6 inhibitors

The CDK4/6-Rb-E2F pathway. CDK4/6 are key regulators of the cell cycle via the CDK4/6-Rb-E2F pathway, with CDK4/6 assuming a pivotal role in the G_1-S checkpoint of the cell cycle, which oversees genome replication (9). The Rb suppressor protein represents a principal cell cycle target of CDK4/6. In normal cells, the phosphorylated state of Rb is typically the consequence of a sequential interaction between CDK4/6 and the D-type cyclin subunit, inhibiting Rb activity (10). The D-type cyclin subunit is generated by stimulating activation signals, including growth factors and cell adhesion molecules. The phosphorylation of Rb results in the deregulation of E2F inhibition, thereby enabling its activation of downstream targets. This, in turn, facilitates the recruitment of transcriptional activators and the irreversible entry of quiescent cells into the DNA-synthesis (S) phase of the cell cycle (1,11). CDK4/6 inhibitors induce complete Rb dephosphorylation, resulting in the sequestration of the transcription factor E2F and subsequent inhibition of cell cycle progression, thereby causing cell cycle arrest in G_1 (12). Common CDK4/6 inhibitors include palbociclib, ribociclib and abemaciclib. The fundamental mechanism of CDK4/6 inhibitors and their role in regulating the cell cycle are elucidated in the present review.

CDK4/6 inhibitors prevent the formation of an active CDK4/6 complex by binding to CDK4/6. The most significant function of cyclin D-CDK4/6 in promoting cellular proliferation is the phosphorylation of Rb, which results in a rapid reduction in the phosphorylation of Rb1 at cyclin D-CDK4/6-dependent sites. This indicates that CDK4/6 is subject to acute inhibition (13). Palbociclib, ribociclib and abemaciclib have been demonstrated to impede the binding of CDK4 and CDK6 to cell division cycle 37, the kinase-targeting subunit of heat shock protein 90 (HSP90). This prevents CDK4/6 from entering the HSP90-chaperone system (14). CDK4/6 inhibitors have demonstrated considerable efficacy in treating $HR^+/HER2^-$ MBC by binding to CDK4/6 (15). In addition to this mechanism, the upregulation of cyclin D expression and formation of the cyclin D-CDK4/6 complex results in the redistribution of kinase inhibitor protein/CDK inhibitor protein inhibitors from the cyclin E-CDK2 complex to cyclin D-CDK4/6, which activates the kinase activity of cyclin E-CDK2 and facilitates cell-cycle progression. This pathway can also be blocked by CDK4/6 inhibitors (13).

The cell cycle is divided into interphase and mitosis (M). Interphase encompasses G_1, S and pre-division (G_2) phases. The cell cycle regulates intricate, orchestrated interactions between cyclin-regulating proteins and cell CDKs (16). CDK4/6 are two major kinases promoting the G_1-S phase transition by phosphorylating and inactivating Rb, facilitating S-phase entry and DNA replication. While the level of CDK remains constant throughout the cell cycle, the expression of cyclin D is subject to dynamic regulation at multiple levels (17). Targeting CDK4 and CDK6 with CDK4/6 inhibitors blocks the binding of cyclin D to CDKs, thereby halting the cell cycle at the G_1 phase and preventing cell proliferation (18). The uncontrolled proliferation of cells, driven by aberrant cell cycle progression, represents a pivotal characteristic of cancerous growth. Consequently, the pursuit of pharmacological agents that impede the cell cycle represents a rational strategy for the treatment of cancer (19). The cell cycle-blocking effect of CDK4/6 inhibitors has markedly benefited the utilization of these pharmaceutical agents in treating cancer (Fig. 1).

The role of estrogen and estrogen receptors (ERs) in regulating the expression of cyclin D. The hormones estrogen and progesterone have been shown to trigger the process of breast morphogenesis through a mechanism of action on a specific subset of mammary epithelial cells that express their cognate receptors, known as ERα and progesterone receptor (PR). Cyclin D1, a critical oncogene in BC, is frequently amplified in ER- and PR-positive BC, which is associated with a poor patient prognosis. Cyclin D1 is specifically amplified in $ER\alpha^+$ BC and is a progesterone target gene (20); thus, the process of progesterone-induced cell proliferation may manifest through the expression of cyclin D1. The binding between PR and progesterone leads to cyclin D1 expression, which is connected to intracellular signaling and activation of the mitotic cell cycle (21). Research findings have demonstrated that in animals injected with $ER\alpha^-$ cells, a substantial decrease in tumor volume is observed following treatment with adiponectin; conversely, in animals injected with $ER\alpha^+$ cells, tumor growth is increased. In the absence of ERα, the process of auto linker-mediated downregulation of cyclin D1 has been shown to involve the recruitment of co-stressors; by contrast, in the presence of ERα, the expression of cyclin D1 was revealed to be induced by the activation complex (22). Cyclin D1 has been demonstrated to regulate PR expression, thereby enhancing responses to estrogen and progesterone. Meanwhile, progesterone can also regulate cyclin D1 through genetic effects or positive feedback loops. It has been demonstrated that estrogen treatment of cyclin D1-transgenic mice can lead to an increase

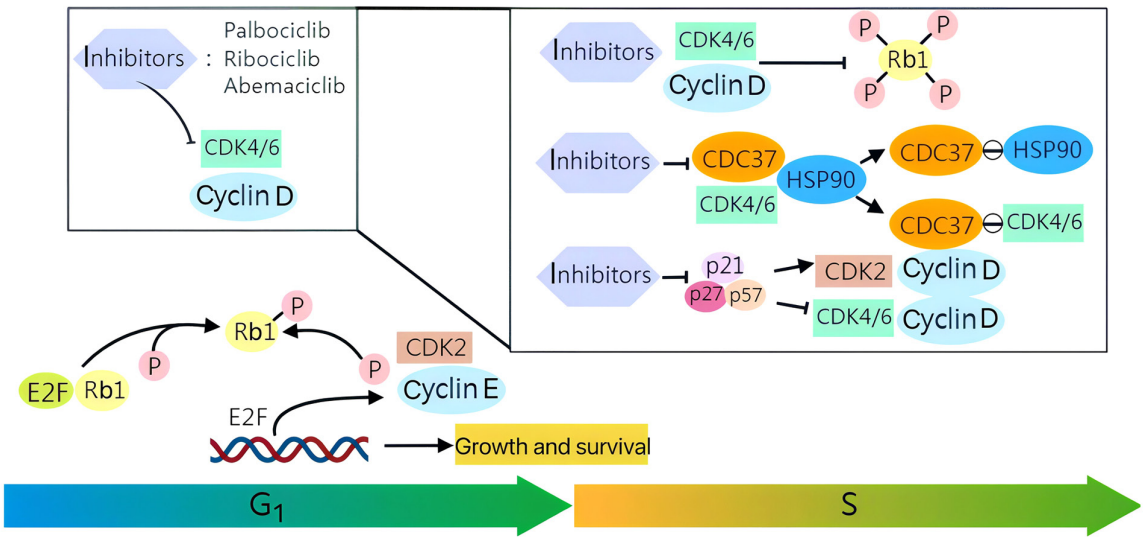

Figure 1. The CDK4/6-Rb-E2F pathway blocks the cell cycle in G_1 phase. Created with MedPeer (medpeer.cn). This diagram illustrates the mechanisms of action of key molecular pathways and related inhibitors in cell cycle regulation. The left side of the diagram illustrates the mechanisms of action of inhibitors, such as palbociclib, ribociclib and abemaciclib, which act on the CDK4/6-cyclin D complex. The right side of the diagram is further refined to present the inhibition of Rb1 phosphorylation by the CDK4/6-cyclin D complex and the interaction of other related proteins, such as CDC37, HSP90, p21, p27, p57 and CDK2, in cell cycle progression. As illustrated in the lower left of the image, E2F is a transcription factor that, under normal conditions, is inactive due to its binding to the Rb1 protein. Upon receiving a growth signal, the CDK2-cyclin E complex is activated, resulting in the activation of CDK2, a cell cycle protein-dependent kinase, through its binding to cyclin E. The activated CDK2-cyclin E complex can phosphorylate the Rb1 protein, causing the dissociation of the phosphorylated Rb1 from E2F. The released E2F is then activated, which in turn initiates the transcription of a series of genes related to growth and survival. These genes drive the cell into specific stages of the cell cycle and promote cell proliferation. The lower arrows signify the mechanism through which CDK4/6 inhibitors impede the progression of cells from the G_1 phase to the S phase, a process that is intricately linked to cell proliferation and survival. CDC37, cell division cycle 37; CDK, cyclin-dependent kinase; HSP90, heat shock protein 90; Rb1, retinoblastoma 1.

in PR expression and the induction of mammary hyperplasia. This process is stimulated by progesterone and blocked by progesterone antagonists. Conversely, this previous study observed a decrease in PR levels in cyclin D1-knockout mice. The mechanism by which cyclin D1 regulates PR expression involves a novel estrogen- and cyclin D1-response enhancer located in the DNA-coding portion of the 3'untranslated region of the PR gene. The expression of PR has been shown to be reduced in human BC cells by small inhibitory RNA which targets at cyclin D1, which also diminishes ER binding to the 3'-enhancing region. Given the established role of estrogen and progesterone in regulating cyclin D1, the involvement of cyclin D1 in the feed-forward loop may potentially increase the risk of BC associated with the combination of estrogen and progesterone (23). Furthermore, the ability of cyclin D1 to regulate PRs suggests a novel function for cyclin D1 in ovarian hormonal control of breast development and breast carcinogenesis (23).

The PI3K/AKT/mTOR pathway of HR$^+$/HER2$^-$ MBC. In addition to CDK4/6-Rb1 signaling, the PI3K/AKT/mTOR signaling pathway is a vital growth stimulation pathway in HR$^+$/HER2$^-$ MBC (24). The PI3K family of proteins is comprised of three classes of lipid kinases that catalyze the phosphorylation of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate. The PI3K class 1 family, a heterodimer with a regulatory and catalytic subunit, is the most commonly involved with human cancer. Somatic mutations in genes encoding components of the PI3K pathway occur in >70% of cases of BC; these include mutations or amplifications of subunit p110α (protein encoded by PIK3CA), subunit p110β and subunit p85α, which are the catalytic subunits of PI3K, or mutations in PI3K modulators, such as phosphatase and tensin homolog (PTEN), AKT and mTOR (25). The most common mechanism underlying PI3K pathway activation is via PIK3CA mutations or amplifications, in which p110α (PIK3CA) serves a vital role in BC tumorigenesis through extra-nuclear ER signaling and is often responsible for endocrine therapy resistance (26). mTOR is a serine/threonine protein kinase and a downstream effector of AKT, which consists of two functionally distinct complexes: mTOR complex (mTORC)1 and mTORC2. mTORC1 belongs to a network of regulatory feedback loops; once activated, it limits the transmission of upstream effectors, such as platelet-derived growth factor receptors α and β, to transmit proliferative signals. This, in turn, results in the attenuation of PI3K/AKT activity. Conversely, mTORC2 is implicated in the regulation of AKT phosphorylation at S473 and the organization of the cellular actin cytoskeleton. Furthermore, mTORC1 activation results in a direct decrease in mTORC2 activity. The mTOR downstream substrate S6 kinase can phosphorylate and activate functional structural domains of ERs, consequently resulting in ligand-independent receptor activation (27). Molecularly, estrogenic activity has been shown to induce insulin-like growth factor, and activate the PI3K/AKT and MAPK pathways, which have been observed to downregulate ERs and PR gene expression at the cell surface. The PI3K/AKT/mTOR pathway is the most frequently altered in MBC, and the upregulation of this pathway has been demonstrated to promote ER-dependent and -independent transcriptional activity, which contributes to anti-estrogen resistance, leading to tumor cell proliferation, survival,

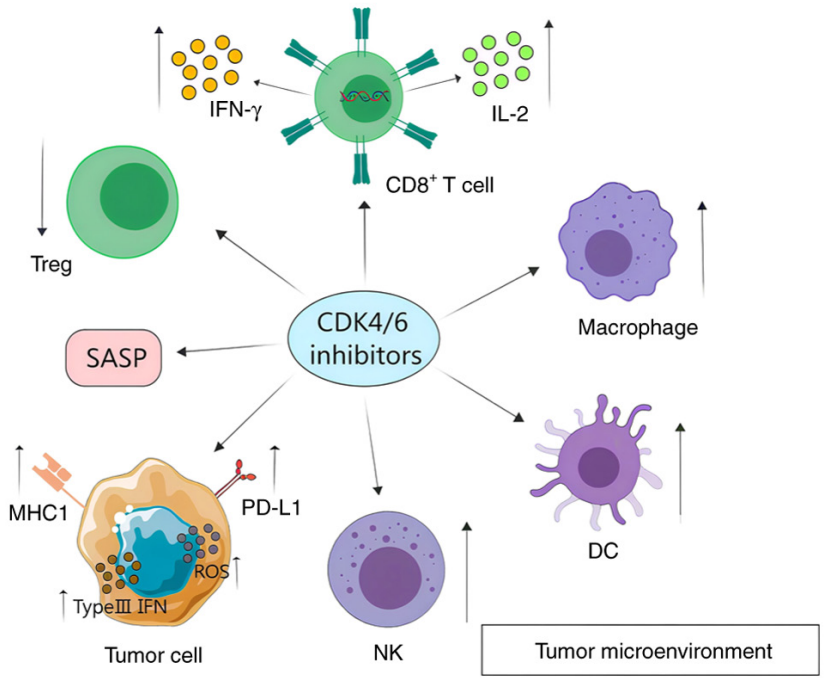

Figure 2. Impact of CDK4/6 inhibitors on the tumor microenvironment. Created with MedPeer (medpeer.cn). This image illustrates the mechanism of action of CDK4/6 inhibitors in the tumor microenvironment. CDK4/6 inhibitors have the capacity to affect a wide range of immune cells and tumor cells. They have been observed to act on $CD8^+$ T cells, prompting them to secrete IFN-γ and IL-2, thereby enhancing immune responses. Additionally, they have been shown to have an inhibitory effect on Tregs, thus regulating immune homeostasis. Regarding tumor cells, the expression of MHC1, PD-L1, ROS and type III IFN is enhanced. Furthermore, they have been observed to enhance the expression of macrophages, DCs and NK cells, among others, thereby remodeling the tumor microenvironment and enhancing the immune surveillance and tumor cell-killing ability. DC, dendritic cell; IFN, interferon; MHC, major histocompatibility complex; NK, natural killer; PD-L1, programmed death-ligand 1; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; Tregs, regulatory T cells.

motility and metabolism. Drugs targeting this pathway have demonstrated encouraging results in $HR^+/HER2^-$ MBC when combined with anti-estrogens (28). In addition, it has been shown that PI3K and mTOR inhibition can enhance sensitivity to endocrine therapies *in vivo*, providing a compelling rationale for the combination of these two therapeutic modalities (29). Similarly, mTORC1/2 inhibitors have demonstrated efficacy in enhancing the sensitivity of drug-resistant HR^+ BC cell lines to CDK4/6 inhibitors (30).

The mechanisms facilitate the understanding of the use of CDK4/6 inhibitors in clinically relevant tumors and in the treatment of $HR^+/HER2^-$ MBC, as mentioned in the present review.

3. Impact of CDK4/6 inhibitors on tumor immunity

CDK4/6 inhibitors affect tumor immunity by altering the immune microenvironment and modulating immune checkpoints (Fig. 2).

Changes in the immune microenvironment. CDK4/6 inhibitors exert a direct effect on tumors by influencing the cell cycle, in addition to affecting cells within the tumor immune microenvironment. CDK4/6 inhibitors enhance cytokine secretion in both tumor cells and cytotoxic T lymphocytes ($CD8^+$ T cells), while the proliferation of immunosuppressive Tregs is inhibited (31). In addition, CDK4/6 inhibitors have been demonstrated to influence the functionality of immune cells, including natural killer (NK) cells and macrophages, which can consequently impact the tumor microenvironment.

T cells. Although CDK4/6 inhibitors have been demonstrated to inhibit T-cell proliferation, they have also been shown to enhance tumor infiltration and effector T-cell activation. Additionally, tumor antigen-experienced T cells demonstrate a heightened sensitivity to CDK4/6 inhibition relative to naive T cells (32). Nuclear factor of activated T cells 4 (NFAT4) is a well-documented regulator of IL-2 secretion, controlled by CDK6, and IL-2 is a well-established marker of cellular activation. It has been demonstrated that brief exposure to CDK4/6 inhibitors impedes CDK6-induced phosphorylation of NFAT4, facilitates its nuclear translocation and augments its transcriptional activity. This ultimately promotes IL-2 synthesis, T-cell activation and cytokine production while reinforcing defense against tumors (33). In a mouse lung cancer model, CDK4/6 inhibitors have been observed to increase the percentage of effector cells within the tumor microenvironment, thereby enhancing the functionality of effector T cells. The capacity of CDK4/6 inhibitors to induce long-term immune T-cell memory in both mouse models and humans provides a robust foundation for treating tumor recurrence (34).

Secondly, it has been observed that CDK4/6 inhibitors exert a considerable inhibitory effect on the proliferation of Tregs. Tregs can impede the proliferation of effector T cells and release cytokines, thereby facilitating tumor cell immune evasion. It has been demonstrated that abemaciclib enhances downstream p21 production via the Rb-E2F axis. p21 can then compete with cyclin D for binding to CDK2, thereby regulating

the G_1-S transition of the cell cycle and mediating cell cycle arrest in Tregs. CDK4/6 inhibitors reduce immunosuppressive Treg populations by inhibiting their proliferation. By inhibiting the Rb-E2F axis and thereby reducing recombinant DNMT1 expression, CDK4/6 inhibitors induce hypomethylation of genes that regulate immune function. Selective inhibition of Treg proliferation may be associated with the observation that Treg cells express higher levels of Rb1 (4,34).

Administration of CDK4/6 inhibitors has been observed to reduce the number of circulating Tregs, the proportion of Tregs in tumors and their cellular activity. Notably, Tregs have been observed to exhibit elevated CDK6 expression relative to other T-cell subtypes (35). This observation suggests that heightened levels of CDK6 and potential increased reliance on CDK6 in Tregs could contribute to their heightened sensitivity to CDK4/6 inhibitors. Furthermore, CDK2 has been demonstrated to function as a negative regulator that CDK4/6 can modulate. CDK4/6 inhibitors influence cells by regulating CDK2 levels in $FOXP3^+$ Tregs, thereby affecting Treg formation (31). In addition, it has been demonstrated that p21 can competitively bind to CDK2, thereby regulating the G_1-S transition of the cell cycle and mediating the cycle block of Tregs. CDK4/6 inhibitors affect Treg cycle block via the Rb-E2F-DNMT1 pathway, inhibiting Treg proliferation, reducing cytotoxic T-lymphocyte depletion and enhancing antitumor immunity (34).

Consequently, CDK4/6 inhibitors can modify the tumor immune microenvironment, namely by enhancing the activation of effector T cells, the capacity to generate immune T-cell memory over time and the inhibition of Tregs proliferation.

NK cells, macrophages and other immune cells. CDK4/6 inhibitors have been demonstrated to enhance antigen presentation. The genes encoding major histocompatibility complex (MHC) class I molecules in cancer cells are upregulated by CDK4/6 inhibitors, thereby strengthening the ability of immune cells to process and present antigens. Inhibition of the CDK-Rb-E2F axis by CDK4/6 inhibitors has been shown to attenuate methylation of endogenous retroviral genes, increase levels of double-stranded RNA and enhance type III interferon (IFN) synthesis. Notably, elevated levels of type III IFN stimulate the expression of IFN-sensitive genes and promote the transcription of downstream genes encoding MHC class I molecules, thereby enhancing the antitumor capacity of immune cells (36). It has been postulated that CDK4/6 inhibitors may exert direct immunomodulatory effects on tumor cells. The number of MHC class I proteins on the surface of tumor cells is increased by genes involved in peptide cleavage and transport. This also enhances the activation of antigen-presenting cells (APCs) and strengthens their ability to deliver antigens (34). Drug-resistant tumors adopt an immunosuppressive microenvironment characterized by elevated MHC II-low macrophages and increased PD-L1 expression on tumor cells. This phenomenon can be partially attributed to low MHC-II levels in macrophages, which result in decreased antigen presentation and T-cell activation (37). CDK4/6 inhibitors have the potential to impede immune evasion by enhancing antigen presentation to resistant tumors, thereby increasing MHC-II levels in macrophages. Concurrently, CDK4/6 inhibitors elevate PD-L1 levels, which counteract the antitumor impact. Consequently, the requirement for concomitant administration of ICIs arises.

The recruitment of NK cells in a senescence-dependent manner has been demonstrated to mediate tumor regression. Cellular senescence is a form of stable cell cycle arrest, and a marked proportion of cancer cells retain the ability to undergo this process. Consequently, the activation of senescence represents a promising new approach to cancer intervention. Palbociclib induces cell cycle arrest and leads to cellular senescence (38). Following dilution or washout from the extracellular medium, palbociclib is released from intracellular acidic vesicles; this reversible drug storage in acidic vesicles is often called lysosomal trapping. It has been demonstrated that brief exposure of cells to palbociclib is sufficient to produce stable cell cycle arrest and long-term senescence (39). NK cells can recognize and eliminate senescent cells that express activating receptors, such as NK cell group 2D and DNAM-1 ligand, by releasing granules containing perforin and granzyme. Combining CDK4/6 inhibitors with a targeted drug (trametinib) and hormone (hepcidin) after endogenous tumor formation has been shown to result in a notable reduction in Rb phosphorylation and tumor cell proliferation, accumulation of SA-β-gal^+ senescent tumor cells, induction of senescence-associated secretory phenotype factors (including TNF-α and intercellular cell adhesion molecule-1) and subsequent NK cell recruitment in a KP GEMM mouse model of lung adenocarcinoma (38).

Serum amyloid A1 (SAA1) serves a notable role in inflating tumor cell senescence induced by palbociclib (4). This acute-phase protein is a serum factor that exerts differential effects on the neutrophil phenotype, enhancing the inflammatory response when neutrophils arrive at the site of injury and promoting resolution during the resolution phase of inflammation (40). Senescent cells coordinate inflammatory processes and their resolution, partly by regulating SAA1, through a coordinated program involving the transition of neutrophils from an 'N1' to an 'N2' phenotype (41). Following tissue injury, induced senescence prevents the propagation of damage by preventing the proliferation of damaged cells (42). Furthermore, it causes tissue remodeling by providing large amounts of bioactive factors in the microenvironment. This perspective elucidates the capacity of senescent cells to engage the immune system to facilitate their clearance while promoting the stem cell signature of surrounding cells to enhance tissue repair (43). Palbociclib confers reversible phenotypic senescence on tumor cells, and it induces a senescent phenotype with an inflammatory secretosome that recruits and activates neutrophils by releasing different inflammatory factors, such as IL-8 and SAA1. Meanwhile, activated neutrophils can cause phagocytosis of senescent tumor cells (44).

Among the various immunotherapeutic strategies, macrophage-based therapies have garnered marked interest in tumor immunotherapy, given that macrophages represent a prominent component of tumor-infiltrating immune cells. CDK4/6 inhibitors can induce differentiation, maturation and antitumor activation of macrophages, which can differentiate into the M1 type (antitumor type). This subsequently opens up immune-suppressive signaling pathways, as evidenced by an increase in the expression of PD-L1 and colony-stimulating factor 1 receptor, and a dependence on lipid metabolism (45).

Macrophages are also implicated in the clearance of senescent cells. The repolarization of macrophage subtypes from M2 to M1 demonstrates a capacity to reverse tumor immunosuppression and promote antitumor immunity within the tumor microenvironment (46). Additionally, macrophages have a prolonged half-life in the circulation and can migrate specifically to tumors. Consequently, macrophages can be utilized as drug carriers for tumor therapy. It has been demonstrated that the selective loading of the photosensitizer black phosphorus quantum dots and abemaciclib into artificially assembled macrophages can confer immunomodulatory and tumor-killing effects within the microenvironment of malignant peripheral nerve sheath tumors (47).

CDK4/6 inhibitors can enhance the antitumor effect of DCs. However the loss of dendritic cells (DCs) within the tumor microenvironment due to the damage by the tumor represents a notable impediment to antitumor immunity following the administration of CDK4/6 inhibitors and immune checkpoint blockade (ICB). Consequently, restoring the DC compartment through the adoptive transfer of *ex vivo*-differentiated DCs has been shown to result in potent tumor suppression in CDK4/6 inhibitors- and ICB-treated patients of BC. The addition of DCs can facilitate tumor localization and induction of systemic CD4 T-cell responses in patients treated with the CDK4/6 inhibitors-ICB-DC combination, which is characterized by enrichment of programmed death-1 (PD-1), and activating phenotype-1-negative T helper (Th)1 and Th2 cells. Depletion of CD4 T cells can abrogate the antitumor benefit of the CDK4/6 inhibitors-ICB-DC combination, and growing tumors exhibit an increased proportion of terminally depleted CD8 T cells. Furthermore, the restoration of DC function through the adoptive transfer of exogenous mature bone marrow-derived DCs has been reported to result in an enhanced tumor response to CDK4/6 inhibitors and ICB therapy. These findings provide substantial evidence that the limited efficacy of CDK4/6 inhibitors and ICB combination therapy observed in clinical trials may be attributable to the inhibitory effect of CDK4/6 inhibitors on DCs. Furthermore, DC therapy may be viable for augmenting CDK4/6 inhibitors and immunotherapy responses in clinical settings (48).

Taken together, CDK4/6 inhibitors can modify the tumor immune microenvironment by enhancing the capacity of immune cells, including NK cells and macrophages, to present antigens, induce tumor cell senescence and recruit neutrophils for tumor destruction. Additionally, they can repolarize macrophage subtypes from M2 to M1, thereby augmenting antitumor activity. Additionally, DC therapy has been demonstrated to enhance the efficacy of CDK4/6 inhibitors.

Immune checkpoint modulation

The combination of CDK4/6 inhibitors with ICIs can enhance antitumor immune responses. The administration of a single CDK4/6 inhibitor has been demonstrated to elevate PD-L1 levels, thereby impeding the response of the immune system to tumor cells. The molecular mechanisms underlying this process have been the subject of investigation. It has been reported that the abundance of the PD-L1 protein is regulated by the cyclin D-CDK4 and cullin 3 speckle-type POZ protein (SPOP) E3 ubiquitin ligase via proteasome-mediated degradation (49). *In vivo*, the inhibition of CDK4/6 has been observed to increase PD-L1 protein levels, mainly by inhibiting cyclin D-CDK4-mediated phosphorylation of SPOP, which in turn promotes SPOP degradation by anaphase-promoting complex/cyclosome bound to the regulatory subunit Cdh1 (49). It has been established that PD-1 is a pivotal immune checkpoint that modulates the threshold of T- and B-cell response to antigens. As a crucial regulatory checkpoint for T cells, PD-1 exerts a central role in governing their cellular function. The interaction between PD-L1 and PD-1 induces T-cell depletion, thereby promoting immune evasion (50). The immunoreceptor tyrosine-based switch motif (ITSM) is an essential site of PD-1 biological function, and PD-1 is phosphorylated by binding to PD-L1 and further induces immunosuppression by activating a series of intracellular pathways. The specific mechanisms by which PD-1 exerts its immunosuppressive effects differ between T and B lymphocytes (51). Two signaling pathways have been identified as being involved in the immune response induced by T cells after pathogen invasion. The first pathway is the binding of MHCs on the surface of APCs to the T-cell receptor (TCR) (52). The second pathway is the binding of immunostimulatory ligands expressed by APCs to the TCR (52). Consequently, this results in the transduction of activating or inhibitory signals to T cells, modulating immune responses such as T-cell activation and exhaustion. The engagement of PD-L1 and PD-1 in T cells leads to the recruitment of Src homology 2-containing tyrosine phosphatase 1/2 (SHP-1/2) to the C-terminus of ITSM. Subsequently, SHP-2 functions by dephosphorylating TCR-associated CD-3ζ and ZAP70, inhibiting downstream signaling. Specifically, the PI3K pathway has been shown to be inhibited, and the expression of the cell survival gene BCL-XL is reduced.

PD-1 has also been shown to impede TCR-induced PI3K/AKT pathway activation by stimulating PTEN. Moreover, PD-1 has been shown to restrict the proliferation of T cells by hindering the activation of the RAS/MEK/ERK pathway. Concurrently, PD-1 suppresses the activation of PKCδ, thereby diminishing the secretion of cytokines, such as IFN-γ and IL-2, by T cells (53). Furthermore, PD-1 signaling orchestrates T-cell metabolism by impeding glycolysis while promoting lipolysis and fatty acid oxidation (54).

The PD-1/PD-L1 interaction has also been demonstrated to inhibit B-cell activation. The binding of PD-L1 to PD-1 results in the phosphorylation of two tyrosines located within the ITSM of PD-L1 by the B-cell receptor. This, in turn, leads to the recruitment of SHP-2 to the C-terminus of PD-1. Subsequently, SHP-2 undergoes phosphorylation. Consequently, the dephosphorylation of SHP-2 by the C-terminus of PD-1 leads to acute Ca^{2+} disruption and long-term growth arrest. Consequently, the PD-1-mediated suppression of the immune response of B cells to antigens is impaired (55).

Notably, combining CDK4/6 inhibitors with PD-1/PD-L1 inhibitors has been demonstrated to augment the antitumor immune response. The antitumor response has been reported to be further enhanced when anti-PD-1 and anti-CTLA-4 are used in combination with CDK4/6 and PI3K inhibitors, resulting in a more robust antitumor effect in humans. This combination therapy can result in enhanced T-cell activation and a reduction in the number of Tregs, thereby enhancing cancer cell death (4). Schaer *et al* (56) demonstrated that the combination of abemaciclib and anti-PD-L1 treatment results

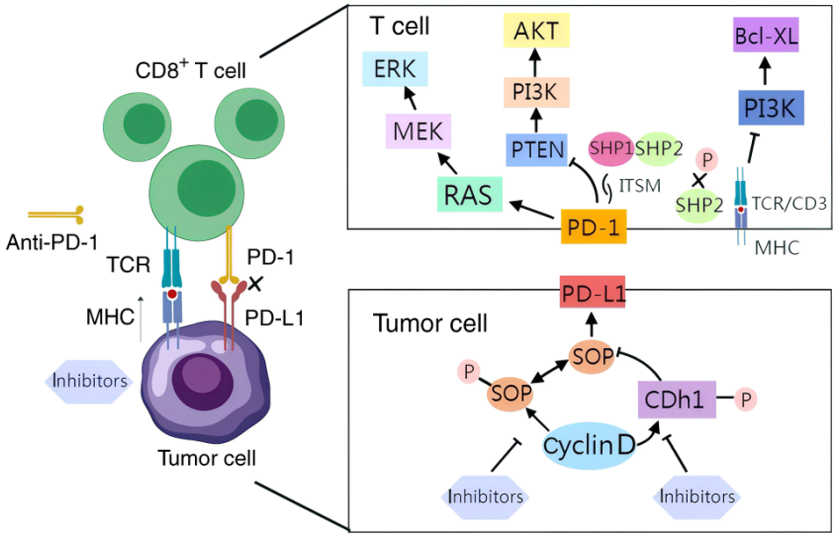

Figure 3. Combining CDK4/6 inhibitors with immune checkpoint (PD-1/PD-L1) inhibitors has been demonstrated to enhance antitumor immune responses. Created with MedPeer (medpeer.cn). As illustrated on the left side of the image, the TCR on the surface of $CD8^+$ T cells recognizes MHC on the tumor cells. Concurrently, PD-1 binds to PD-L1 on the tumor cells, thereby inhibiting T-cell activity. This inhibitory signaling can be blocked with anti-PD-1 drugs. The upper right part of the diagram illustrates the signaling pathways in T cells, including the RAS/MEK/ERK and PI3K/AKT pathways, as well as inhibitory signaling through SHP1 and SHP2 after PD-1 activation. The lower right part of the diagram illustrates the signaling pathways within tumor cells. Notably, PD-L1-related signals have the capacity to influence various critical processes within tumor cells, such as the proliferation of tumor cells and the regulation of other processes. This influence can be mitigated by the intervention of related inhibitors within the signaling pathways. MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PTEN, phosphatase and tensin homolog; SHP, Src homology 2-containing tyrosine phosphatase; TCR, T-cell receptor.

in the increased expression of genes involved in antigen presentation and the innate immune response, which in turn leads to an increase in the expression of MHC class I and class II molecules in tumor cells. In a BC mouse model, Goel *et al* (4) demonstrated that the combination of CDK4/6 inhibitors and PD-1/PD-L1 inhibitors directly activates T cells and modulates innate immunity by enhancing antigen presentation. The combination therapy has been demonstrated to present superior efficacy in inhibiting tumor growth compared with abemaciclib monotherapy. Furthermore, combining CDK4/6 inhibitors with anti-PD-1 antibodies can enhance tumor control in most mouse models (34). Preclinical *in vivo* BC studies have indicated that CDK4/6 inhibitors may enhance tumor sensitivity to PD-1/PD-L1 blockers. Notably, Schaer *et al* observed that treatment with a CDK4/6 inhibitor followed by the staged use of anti-PD-L1 agents results in more complete remission (31). In conclusion, further investigation into the potential of combining CDK4/6 inhibitors with ICIs to enhance antitumor immune responses is warranted (Fig. 3).

The impact of CDK4/6 inhibitors on immune evasion within the tumor microenvironment. PD-1 on immune cells and PD-L1 on tumor cells can facilitate immune escape. The expression of PD-L1 on tumor cells can be promoted by CDK4/6 inhibitors, which may encourage immune evasion in a variety of tumor models (34). CDK4/6 inhibitors have been demonstrated to elevate PD-L1 levels, thereby enhancing the interaction of PD-L1 with the PD-1 receptor on the surface of T cells and inhibiting T-cell activity (57); this allows tumor cells to evade the immune system. Concurrently, CDK4/6 inhibitors also elevate the number of negative immunodetection sites, PD-1 and CTLA-4, on T lymphocytes, thereby exerting an adverse immunomodulatory influence. However, CDK4/6 inhibitors can also induce cell cycle blockade, trigger T cells, enhance antigen presentation, stimulate the immune system, inhibit the growth of Tregs and reduce the secretion of inhibitory cytokines. This results in a decrease in the inhibitory effect on immune cells and an increase in the immune response against tumor cells. Consequently, the impact of CDK4/6 inhibitors on immune evasion within the tumor microenvironment is multifaceted. It is of the utmost importance to exercise caution when developing antitumor drugs that utilize CDK4/6 inhibitors to prevent immune evasion by tumor cells.

4. Current research progress on CDK4/6 inhibitors

Approved CDK4/6 inhibitors. Given the prevalence of cell cycle gene abnormalities in solid tumors, developing and approving CDK4/6 inhibitors for treating several solid tumors in recent years has been a significant advancement in oncological therapy. This section lists the most used CDK4/6 inhibitors approved for clinical use.

Palbociclib (Ibrance®; Pfizer) was first synthesized in 2001 and was the original CDK4/6-specific inhibitor. In February 2015, the U.S. Food and Drug Administration (FDA) granted accelerated approval of palbociclib for postmenopausal women with advanced or metastatic HR^+/HER^- MBC as a first-line therapy (58). Palbociclib was approved for use with aromatase inhibitors (AIs) based on PALOMA-1 and PALOMA-2 trial findings (6,59). Palbociclib was also approved in combination with fulvestrant based on the results of the PALOMA-3 trial (60). In 2019, the FDA expanded the use of palbociclib in combination with an AIs or fulvestrant to include men (61). The agent in question has been demonstrated

to reduce the proliferation of ER-positive BC cell lines *in vitro* by blocking Rb phosphorylation, leading to G_1 phase arrest (62).

Ribociclib (Kisqali®; Novartis), another oral CDK4/6 small-molecule inhibitor, was approved by the US FDA in March 2017 in combination with AIs for first-line endocrine therapy in postmenopausal patients with HR^+/HER^- MBC. This is based on the progression-free survival (PFS) and overall survival (OS) benefits demonstrated with ribociclib in the MONALEESA program (63-65). On July 18, 2018, the FDA expanded its indications and approved ribociclib combined with fulvestrant for treating postmenopausal patients with HR^+/HER^- MBC. On the same day, the FDA also approved ribociclib in combination with AIs as initial endocrine therapy for premenopausal, perimenopausal and postmenopausal patients with $HR^+/HER2^-$ MBC (66). On September 17, 2024, based on the results of the NATALEE study (67), the FDA approved ribociclib in combination with AIs as adjuvant therapy for $HR^+/HER2^-$ stage II and III early BC in adults with a high risk of recurrence.

Abemaciclib (Verzenios®; Eli Lilly and Company) was approved by the FDA in September 2017 in combination with fulvestrant as initial endocrine therapy based on the results of the MONARCH-2 trial (68); it is suitable for postmenopausal patients with $HR^+/HER2^-$ MBC. In February 2018, based on the results of the MONARCH-3 study, the FDA approved abemaciclib for combination therapy with AIs (69). In addition, the MONARCH-1 study showed the benefit of abemaciclib as monotherapy in severely pretreated patients (70). Abemaciclib is the first CDK4/6 inhibitor approved for $HR^+/HER2^-$ early-stage BC with a high recurrence risk (71). The FDA approved abemaciclib in combination with endocrine therapy (tamoxifen or AIs) in October 2021 as adjuvant therapy for adult patients with $HR^+/HER2^-$, node-positive, early-stage BC with a high risk for recurrence and a Ki-67 score ≥20% (72). In March 2023, the FDA expanded the indications for abemaciclib, eliminating the requirement for a Ki-67 score (73).

In three large randomized phase III trials, the CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib were investigated when each was combined with AIs in the first-line treatment of postmenopausal patients with $HR^+/HER2^-$ MBC (59,65,69). The three CDK4/6 inhibitors resulted in marked increased in PFS compared with AI treatment alone, exhibiting nearly identical hazard ratios for PFS and potentially analogous efficacy. In another three other large randomized phase III trials, PALOMA-3 (palbociclib), MONALEESA-3 (ribociclib) and MONARCH-2 (abemaciclib), the combinations of the three CDK4/6 inhibitors with fulvestrant were investigated in patients with $HR^+/HER2^-$ MBC as the second line of therapy (63,74,75). In the CDK4/6 inhibitor group, all three drugs showed prolonged PFS and OS benefits compared with fulvestrant alone. The results from pertinent clinical studies are provided in Table SI.

Palbociclib, ribociclib and abemaciclib have been widely used in clinical practice, mostly in combination with endocrine therapy for $HR^+/HER2^-$ MBC. In addition, extensive comparative studies on the efficacy and safety of these three drugs have been conducted. Desnoyers *et al* (76) performed a meta-analysis, which showed that the OS and PFS of the three drugs in the treatment of patients with $HR^+/HER2^-$ MBC are similar, and supplemented the relative safety and tolerability of the three drugs, which may help guide clinicians in the decision-making of drug selection. The results of this previous study showed that ribociclib and abemaciclib are associated with significantly lower rates of grade 3-4 neutropenia, but significantly higher gastrointestinal toxicity when compared with palbociclib. In addition, abemaciclib is less well tolerated, and patients treated with this drug were reported to have a significantly higher incidence of treatment interruptions due to adverse events (AEs) compared with the other two drugs. In 2024, a meta-analysis by Kappel *et al* (77) also showed similar results. Some additional findings from this study were that palbociclib was shown to be associated with a significantly higher incidence of neutropenia, but a substantially lower risk of grade 3-4 infections than ribociclib and abemaciclib. Cases of grade 3-4 transaminitis and grade 3-4 neutropenia were also reported to be significantly lower with abemaciclib than with ribociclib.

CDK4/6 inhibitors in clinical trials. The preliminary preclinical data indicate that BC, particularly the HR^+ form, is highly susceptible to CDK4/6 inhibitors. Consequently, numerous clinical trials have concentrated on this specific cancer type (6,78,79). A substantial body of clinical evidence supports the use of CDK4/6 inhibitors in the treatment of postmenopausal women with advanced or $HR^+/HER2^-$ MBC, demonstrating a marked prolongation of patient survival (74,78,79). Consequently, the FDA has approved several CDK4/6 inhibitors in clinical settings, including palbociclib, ribociclib and abemaciclib. In recent years, a number of novel CDK4/6 inhibitors have commenced clinical investigation. Table SII provides an overview of the drugs subjected to clinical studies (80-84).

G1T38 is a novel CDK4/6 inhibitor developed by G1 Therapeutics, Inc. G1T38 has been demonstrated to block cells in the G_1 phase by reducing Rb phosphorylation and inhibiting cell proliferation in CDK4/6-dependent oncogenic cell lines, including those derived from BC, melanoma and leukemia. Furthermore, G1T38 has been shown to possess selective and oral bioavailability characteristics, with good selectivity. It is currently undergoing phase II clinical trials and may become an effective CDK4/6 inhibitor in the clinic in the future (81).

Narazaciclib (ON123300) is a CDK4/6 inhibitor developed by Onconova Therapeutics, Inc. and is undergoing phase I/II clinical studies. A previous study has shown that it exhibits relatively high antitumor activity in mantle cell lymphoma cell lines (82).

PF-06873600, a CDK2/4/6 inhibitor developed by Pfizer, has been identified as a potential therapeutic agent for cancer treatment and has been subjected to phase I clinical trials for the treatment of patients with $HR^+/HER2^-$ BC, metastatic triple-negative BC (TNBC) or advanced cisplatin-resistant epithelial ovarian/tubal carcinoma (83).

ETH-155008, developed by Shengke Pharmaceutical (Jiangsu) Ltd., represents a novel dual inhibitor that may enable effective acute myeloid leukemia (AML) therapy through dual targeting of FLT3 and CDK4/6. By further blocking tumor cells at the G_1 stage, ETH-155008, through a synergistic effect, to some extent avoids the development of CDK4/6 inhibitor resistance. Notably, it shows a favorable safety and efficacy profile in phase I clinical studies (84).

AMG925 (FLX925), developed by Amgen, has been shown to act on FLT3 and CDK4/6 selectively. It is currently in phase I clinical trials, primarily for the treatment of AML (80).

In addition to new CDK4/6 inhibitors entering clinical studies, various drugs approved for clinical use, including palbociclib, ribociclib and abemaciclib, are also under investigation. In addition to HR^+ BC, the clinical studies of these drugs have primarily focused on other solid tumors, including non-small cell lung cancer and prostate cancer. In addition, further in-depth research may be conducted on the therapeutic effect, safety, pharmacokinetics, dosage regimen and other pertinent factors of these drugs. Table SIII provides a comprehensive listing of clinical trials related to CDK4/6 inhibitors, as listed on ClinicalTrials.gov (85-92).

5. Mechanism of CDK4/6 inhibitors resistance

Resistance to CDK4/6 inhibitors is an almost inevitable biological event. From a therapeutic point of view, it has become urgent to find biomarkers of sensitivity and resistance, to understand the mechanisms of resistance to CDK4/6 inhibitors, and to identify strategies to overcome them according to the target. A trial of patients with $HR^+/HER2^+$ MBC treated with a combination of CDK4/6 inhibitors and endocrine therapy, and analyzed by biopsy whole exome sequencing, identified eight different mechanisms of CDK4/6 inhibitor resistance (93): i) Rb1 deletion, ii) AKT1 mutation/amplification, iii) RAS activation, iv) fibroblast growth factor receptor (FGFR) activation, v) HER2 activation, vi) cell cycle protein-E activation, vii) Aurora kinase A (AURKA) amplification and viii) estrogen signaling deletion. These diverse mechanisms (including alterations in cell cycle-specific or non-specific mechanisms) have been observed in two-thirds of resistant tumors examined. These mechanisms can be further subdivided into the following categories: Rb function deficiency, abnormalities in cell cycle regulatory factors, activation of bypass signaling pathways, and dysfunction of other transcription factors and hormones. The identification of these mechanisms has the potential to yield novel therapeutic interventions for patients with these tumors.

Alterations in specific mechanisms of the cell cycle

Rb deletion and E2F amplification. CDK4/6 inhibitors inhibit cell proliferation through a mechanism of action dependent on Rb. Specifically, CDK4/6 inhibitors block CDK4/6 activity and prevent phosphorylation of the Rb protein, which in turn maintains the inhibition of Rb on the E2F transcription factor and prevents the cell cycle from moving from the G_1 phase into the S phase (94,95). However, it has been reported that a variety of BC cell lines resistant to palbociclib possess reduced levels of Rb expression compared with cells that had not received the drug (96). This implies that CDK4/6 inhibitors treatment may reduce Rb protein levels while inhibiting proliferation. This phenomenon has been further validated in clinical practice, where up to 10% of patients with disease progression treated with CDK4/6 inhibitors have been shown to possess alterations or deletions in both copies of the Rb1 gene, a feature not seen in patients treated with endocrine therapy only (97). Rb1 loss is a proven resistance mechanism *in vitro* and in patient-derived tumor xenograft models (24). The level of Rb1 protein decline is further exacerbated by multiple molecular causes (such as mutation, silencing or hyperphosphorylation) (98). Ultimately, the constitutive progression of the cell cycle continues through the activation of other cell cycle mechanisms (such as E2F amplification) and is not driven in a manner that relies on CDK4/6 (99). However, a simple decline in Rb levels is not sufficient to activate E2F transcriptional activity necessary for cell cycle progression, nor is it alone adequate to directly drive cell cycle deregulation (100). In the process of developing CDK4/6 inhibitor resistance, upregulation of mitogenic or hormonal signaling, or activating mutations are also required to stabilize c-Myc levels, which mediate the amplification of E2F transcriptional activity, bypassing the inhibitory effects of CDK4/6 inhibitors (96). Therefore, for this group of patients, restoring their Rb levels or inhibiting c-Myc to reduce E2F activity may be two critical methods to overcome drug resistance.

Abnormalities in cell cycle regulators

Amplification of CDK4/6. Amplification of CDK4 or CDK6 is considered one of the essential mechanisms of CDK4/6 inhibitor resistance, as this amplification can drive cell cycle progression through activation of the cyclin D-CDK4/6-Rb pathway, thereby impairing the effect of CDK4/6 inhibitors on cell cycle arrest (101). CDK4 is widely expressed in various cancer types and CDK4-upregulated cells have reduced sensitivity to CDK4/6 inhibitors. In glioma cells, CDK4 overexpression can even lead to complete resistance to CDK4/6 inhibitors (102). In addition, CDK4 overexpression has been identified as a potential biomarker for predicting resistance to conventional chemotherapy in patients with osteosarcoma (103). Moreover, the overexpression of CDK4 may be attributed to the enhancement of CDK4 activation by the cell-cycle regulator CDK7 through T-loop phosphorylation (104). Coombes *et al* (105) reported that the CDK7 inhibitor samuraciclib exerts some clinical activity with an objective remission rate of 36% (9/25 patients with $HR^+/HER2^-$ BC). In a population of patients previously treated with a CDK4/6 inhibitor in combination with fluvastatin, samuraciclib was associated with a median PFS time of 3.7 months (105). These data suggest that samuraciclib may have potential clinical benefit in managing disease progression associated with CDK4/6 inhibitor resistance (105).

It has also been reported that the CDK6 gene locus is *de novo* amplified in a cellular model of resistance to palbociclib. By contrast, knockdown of CDK6 can restore sensitivity to CDK4/6 inhibitors, suggesting that the level of CDK6 activity also serves a critical role in CDK4/6 inhibitor resistance (106,107). Multiple mechanisms may lead to elevated CDK6 levels. On one hand, mutations in the upstream gene FAT1, and accumulation of YAP and TAZ transcription factors at the CDK6 promoter, lead to Hippo pathway-mediated CDK6 upregulation, which promotes resistance to CDK4/6 inhibition (108). On the other hand, Cornell *et al* (109) reported that, in BC, increased expression of the microRNA (miR)-432-5p exosome may downregulate SMAD4 to weaken the TGF-β signaling pathway, thereby upregulating the expression level of CDK6. During this process, the development of resistance to CDK4/6 inhibitors is due to the highly expressed CDK6 enhancing the activity of the cyclin D-CDK6 complex,

phosphorylating Rb protein, driving the cell cycle process and bypassing the blocking effect of CDK4/6 inhibitors. In addition, miR-432-5p may be delivered by exosomes and can thus diffuse the phenotype of CDK6 upregulation in surrounding cancer cells, which further enhances the drug resistance of the population.

Hyperactivation of the cyclin E-CDK2 complex. The cyclin E-CDK2 complex is another key regulator of the G_1/S phase transition and is capable of phosphorylating the Rb protein independently (110). Notably, amplification of cyclin E1 and cyclin E2 leads to increased CDK2 activity, which in turn promotes the degradation of the CDK inhibitor p27Kip1, thereby facilitating cell cycle progression (111). Under treatment with CDK4/6 inhibitors, aberrant activation of cyclin E and of the CDK2 complex downstream of CDK4/6 can bypass the inhibitory effects of CDK4/6, resulting in complete phosphorylation of Rb and the release of E2F, which triggers the subsequent cell entry into S phase (112,113). Herrera-Abreu *et al* (24) reported that amplification or overexpression of the cyclin E1 gene is significantly associated with palbociclib resistance and that knockdown of cyclin E1 restores cellular sensitivity to this inhibitor. In another correlative analysis of the PALOMA-3 trial (114), 302 patients underwent tumor tissue analysis. The results showed lower efficacy of palbociclib in patients with high vs. low CNE1 mRNA expression (median PFS: palbociclib group, 7.6 vs. 14.1 months; placebo group, 4.0 vs. 4.8 months). In addition, the preoperative palbociclib randomized clinical trial independently validated the role of high cyclin E1 mRNA as a palbociclib resistance marker in regulating Rb phosphorylation and antiproliferative response to palbociclib. Therefore, overexpression of cyclin E is considered one of the potential mechanisms of CDK4/6 inhibitor resistance. Notably, the overexpression of cyclin E was shown to disappear when CDK2 is inhibited, a finding that suggests that the combined CDK2/4/6 inhibition strategy may inhibit cell proliferation through a synergistic effect, thus providing a potential therapeutic tool for the prevention of resistance to CDK4/6 inhibitors (115).

In addition to the cyclin E complex, Cai *et al* (19) showed that the long noncoding RNA (lncRNA) EILA, which interacts with cyclin E1, is upregulated in CDK4/6 inhibitor-resistant BC cells and promotes CDK4/6 inhibitor resistance by stabilizing cyclin E1. By contrast, silencing EILA reduces cyclin E1 protein and restores CDK4/6 inhibitor sensitivity *in vitro* and *in vivo* (19). lncRNAs may thus serve as another potential therapeutic target for overcoming drug resistance.

AURKA amplification. AURKA is a kinase that serves a vital role in cell division, and has been associated with intrinsic and acquired resistance to CDK4/6 inhibitors and endocrine therapies (93). AURKA amplification promotes cell cycle progression directly or indirectly. For example, AURKA can phosphorylate Rb proteins, weakening their inhibitory effect on E2F transcription factors, thereby restoring E2F transcriptional activity and driving the cell cycle from G_1 to S phase (116). In addition, AURKA is a key regulatory component of the p53 pathway, particularly in the checkpoint-response pathway necessary for oncogenic transformation through phosphorylation and stabilization of p53, which confers tumor cell resistance to CDK4/6 inhibitors (117). Together, these mechanisms diminish the cell cycle-blocking effects of CDK4/6 inhibitors. AURKA amplification is also highly associated with CDK4/6 inhibitor treatment failure in clinical samples. Wander *et al* (93) demonstrated that the proportion of AURKA overexpression tends to be higher in patients with disease progression after treatment with CDK4/6 inhibitors than in patients with effective treatment. This finding suggests that AURKA amplification may serve as a potential biomarker of CDK4/6 inhibitor resistance to inform patient stratification and treatment decisions.

Alterations in nonspecific mechanisms of the cell cycle

Activation of bypass signaling pathways

Activation of FGFR1. The FGFR family consists of highly conserved transmembrane receptors (FGFR1-4) involved in a number of key mechanisms that lead to cancer, such as proliferation, differentiation and cell survival (118). Post-progression analysis of circulating tumor DNA from 34 patients treated with palbociclib in combination with letrozole has been reported to show alterations in FGFR, including FGFR1 amplification, FGFR2 amplification, and activating FGFR1 and FGFR2 mutations (119). Amplification of FGFR1 is the most common genomic alteration in BC, occurring in ~10% of BC cases (120), and FGFR1 has been identified as a potential mechanism of acquired resistance to endocrine therapy in combination with CDK4/6 inhibitors (121,122). The development of this resistance mechanism may be mediated by FGFR1 amplification, which activates the PI3K/AKT/mTOR and RAS/MEK/ERK signaling pathways (123). Genetic analysis of the MONALEESA-2 study showed that patients with amplification of the FGFR1/ZNF703 fusion gene had a PFS of 10.61 months, compared with 24.84 months for patients with wild-type FGFR1/ZNF703. In addition, patients with high FGFR1 mRNA expression (50% of the median mRNA expression) had a median PFS time of 22.21 months, whereas patients with low FGFR1 mRNA expression did not reach a median PFS after 32 months of follow-up (65). These results indicate that FGFR1 amplification and high expression may be predictors for a poor prognosis in patients, and CDK4/6 inhibitors combined with blocking the FGFR pathway may be a potential strategy used to overcome drug resistance and improve the prognosis of patients.

Aberrant PI3K/AKT/mTOR signaling. PI3K/AKT/mTOR is hyperactivated or altered in numerous types of cancer and regulates a wide range of cellular processes, including proliferation, growth, metabolism, angiogenesis and metastasis (124). In BC, inhibition of CDK4/6 promotes the adaptive rewiring of the PI3K/AKT/mTOR signaling pathway in BC cells, which enhances their dependence on this axis, promoting phosphorylation and activity of the PI3K/AKT/mTOR pathway (101). Notably, overactivation of the PI3K/AKT/mTOR pathway induces cyclin D1 and CDK4 protein upregulation, whereas direct inhibition of the signaling pathway silences cyclin D1 and CDK4, and leads to cell cycle arrest. These findings confirm that activation of the PI3K/AKT/mTOR pathway and resistance to CDK4/6 inhibitors may be interrelated (125). On the other hand, elevated levels of phosphorylated AKT have been associated with prolonged expression of the cyclin E2-CDK2 complex of cell cycle proteins, which suggests that tumor cells can bypass the cyclin D-CDK4/6-dependent

mechanism of cell cycle regulation via an alternative pathway to maintain cell cycle progression (24).

Abnormal MAPK signaling pathway. The MAPK signaling pathway, or RAS/MEK/ERK pathway, is a signaling pathway downstream of FGFR1. Activation of the RAS family of oncogenes (KRAS, HRAS, NRAS) has been observed in tumor biopsies collected from patients with HR^+ BC resistant to CDK4/6 inhibitors (93). Most patients with KRAS-mutant malignancies exhibit elevated levels of expression of cyclin D1 (126). Patients with high levels of cyclin D1 protein expression tend to have a worse prognosis than those with low expression levels (median OS 41.7 vs. 3.5 months) (127). This may be explained by the fact that KRAS produces aberrant growth signals in the presence of CDK4/6 inhibitors, leading to cellular resistance. In addition, KRAS and RAF kinases regulate ERK1/2 via MEK. ERK1/2 is also highly represented in the oncogenic profile of patient RNAseq data related to the occurrence and development of cancer (128). Based on this, there have been several clinically tested pharmacological agents that target the MAPK pathway. For example, the FDA has approved MEK inhibitors for certain types of cancer (such as melanoma) (128). In BC, the MEK1/2 inhibitor selumetinib, in combination with fulvestrant and palbociclib, can effectively inhibit the proliferation of BC cells resistant to CDK4/6 inhibitors (119). These trials have shown an association between acquired resistance to CDK4/6 inhibitors and induced MAPK pathway activity, indicating that selecting the correct target for targeted therapy is an effective measure to address resistance.

Abnormal expression of other cytokines and hormones. In preclinical models, acquired CDK6 amplification not only confers abemaciclib resistance by bypassing the activation of alternative cell cycle checkpoints, but also leads to a notable reduction in the mRNA and protein levels of ER and PR (106,111). Furthermore, it can suppress the expression of ER-regulated genes, such as XBP1 and MYC (106). Reduced ER/PR levels impair estrogen-dependent induction of cyclinD-CDK4/6 complex activity (129). Notably, the absence of ER/PR expression is closely associated with acquired insensitivity to hormonal agents, such as the ER antagonists fulvestrant and tamoxifen (130), suggesting that the dual mechanisms of drug resistance involve cell cycle dysregulation and endocrine therapy escape.

The AP-1 family is a key transcription factor for cyclin D1, and includes Jun, Fos, and their related subfamilies of activating transcription factors (ATF2, LRF1/ATF3 and B-ATF) (131). In 20-40% of human patients with BC, c-Jun exhibits high levels of activation. It has been reported that c-Jun activation inhibits ER activity and upregulates cyclin D1 transcription, thereby inducing resistance to CDK4/6 inhibitors (132). To the best of our knowledge, the efficacy of AP-1 inhibitors in patients with CDK4/6 inhibitor-resistant BC has not yet been thoroughly investigated. However, the results of this exploration are of great clinical value.

MYC is a widespread oncogenic transcription factor that promotes cancer cell progression (133). MYC-acquired mutations have been identified in 8.8 and 20.1% of patients with BC in phase III MONARCH-3 (NCT02246621) and phase II Next MONARCH-1 clinical trials (NCT02747004) respectively (134,135). The Next MONARCH-1 clinical trial has shown that acquired MYC genomic aberrations were enhanced after abemaciclib monotherapy or combination therapy with a nonsteroidal AI in patients with HR^+ BC (136). In addition, Pandey *et al* (137) has validated the upregulation of MYC in palbociclib-resistant cells by reverse transcription-quantitative PCR, suggesting that the MYC upregulation may be another marker of acquired resistance to CDK4/6 inhibitors.

Despite the diversity of drug resistance mechanisms, there are some commonalities and cross-regulatory relationships among different drug resistance mechanisms. The commonality of such a variety of drug resistance mechanisms lies in the imbalance of the cell cycle regulation system. Whether through loss of Rb function, amplification of CDK4/6 or activation of the cyclin E-CDK2 complex, this ultimately leads to the bypass of the G_1-phase blocking signal. This co-imbalance makes the cells no longer dependent on the CDK4/6-driven pathway, thus reducing the effectiveness of CDK4/6 inhibitors (138). In addition, FGFR1 activation and the abnormal transduction of upstream growth factor signaling pathways (such as PI3K/AKT/mTOR and RAS/MAPK) affected by FGFR1 can not only directly compensate for the loss of cell cycle regulation, but also often interact with the regulation of intracellular cyclin expression. This means that even when CDK4/6 is inhibited, these alternative pathways can activate downstream cell proliferation mechanisms (139). Part of the resistance mechanism, while largely attributable to genetic mutations (such as loss of Rb), is often also accompanied by extensive epigenetic regulatory changes, such as the regulation of AP-1 and MYC transcription factor activity. This interaction at the genetic and epigenetic levels further underlies the commonality between drug resistance mechanisms (140). These commonalities also suggest that drug resistance can be predicted in a timely manner by monitoring Rb status, cyclin E-CDK2 activity and related signaling pathway changes in the early stage, so as to provide personalized treatment. Combined treatment of CDK4/6 inhibitors with endocrine therapy, chemotherapy and targeted therapy can simultaneously target multiple pathways and may effectively overcome the problem of drug resistance.

6. Combination therapy with CDK4/6 inhibitors

Targeting CDK4/6 is under active research in cancer therapy as a promising cancer treatment. The present study previously reviewed the resistance to CDK4/6 inhibitors in cancer therapy, and addressing the resistance to CDK4/6 inhibitors has profound implications for realizing their potential in cancer therapy. Effective combination therapies involving CDK4/6 inhibitors have been shown to delay resistance while reducing the side effects of other treatments, leading to favorable antitumor effects (138). Despite the current lack of effective remedies for low Rb1 expression, which is the most fundamental cause of resistance, several drug combination strategies have shown the potential to improve the drug sensitivity of CDK4/6 inhibitors (141). Drug combination strategies involving CDK4/6 inhibitors may include endocrine therapy, chemotherapy, targeted therapy and immunotherapy.

CDK4/6 inhibitors in combination with endocrine therapy. Endocrine therapy is one of the main treatments for HR^+ BC, and developing novel endocrine therapies in combination with

CDK4/6 inhibitors offers new hope for patients with BC and other types of cancer (142). As aforementioned, palbociclib, ribociclib and abemaciclib were approved by the FDA for HR^+/HER^- MBC based on multiple clinical studies combined with AIs or fulvestrant. In 2017, the PALOMA-2 clinical trial (NCT01740427) by Finn *et al* (143) showed that the combination of letrozole and a CDK4/6 inhibitor (PFS, 27.6 months) prolonged PFS by ~13.1 months compared with letrozole alone (PFS, 14.5 months). For selective ER degraders (SERDs), a clinical trial in advanced BC (NCT01942135) showed that the combination of fulvestrant and a CDK4/6 inhibitor (PFS, 34.9 months) prolonged PFS by 5.2 months compared with fulvestrant alone (PFS, 29.7 months) (75). Another phase II clinical study, MAINTAIN (NCT02632045), had similar results (144). The phase III EMERALD trial (NCT03778931) by Bidard *et al* (145) and the phase II SERENA-2 trial by (NCT04214288) Oliveira *et al* (146) showed that SERD management has a PFS benefit. On January 27, 2023, the next-generation SERD elacestrant was approved by the FDA for patients with metastatic HR^+ BC and ESR1 mutations (147). Notably, two ongoing clinical trials are testing the selective androgen receptor modulator enobosarm alone (NCT04869943) and in combination with abemaciclib (NCT05065411) in patients with $ER^+/HER2^-$ MBC (138).

Currently, most international guidelines recommend first-line CDK4/6 inhibitors for patients with newly diagnosed $HR^+/HER2^-$ MBC (148). However, the optimal timing of CDK4/6 inhibitors has not been fully elucidated. The randomized phase III SONIA trial (NCT03425838), conducted in the Netherlands, randomized 1,050 patients who had not previously received treatment for advanced BC to receive a CDK4/6 inhibitor in either the first-line or second-line setting. This study aimed to address whether delaying CDK4/6 inhibitors to second-line therapy after initial endocrine therapy monotherapy affects efficacy, and also assessed safety, quality of life and cost-effectiveness (149). The study aimed to assess whether all patients should start treatment with CDK4/6 inhibitors. The findings indicated that more research is required to identify patients who truly benefit from first-line CDK4/6 inhibitor therapy and those who do better when deferred to second-line treatment. More recently, Sonke *et al* (150) found no statistically significant benefit of using CDK4/6 inhibitors as first-line therapy compared with second-line therapy (median 31.0 and 26.8 months, respectively; hazard ratio, 0.87; 95% confidence interval, 0.74-1.03; P=0.10). Health-related quality of life was also similar in the two groups. In addition, first-line CDK4/6 inhibitor use was associated with a longer duration of CDK4/6 inhibitor treatment and more grade ≥3 AEs than second-line use. This study challenges past perceptions of the need for first-line use of CDK4/6 inhibitors and points to the need for robust sequencing trial data to determine the best use of available therapies.

Combination chemotherapy. For patients with $HR^+/HER2^-$ BC resistant to endocrine therapy in combination with CDK4/6 inhibitors, the application of combination chemotherapy with CDK4/6 inhibitors may be considered (151). Recently, in a phase III TROPiCS-02 trial (NCT03901339) conducted by Rugo *et al* (152), patients with $ER^+/HER2^-$ BC were evaluated for the efficacy of chemotherapy after CDK4/6 inhibitor resistance. Chemotherapy was indicated to be a viable treatment option after these patients demonstrated resistance to CDK4/6 inhibitors combined with endocrine therapy.

The commonly used chemotherapeutic agent paclitaxel regulates microtubule proteins during cell cycle division, while CDK4/6 inhibitors prevent phosphorylation of Rb1 during the G_0 phase of the cell cycle (153). The combination of paclitaxel and CDK4/6 inhibitors can co-regulate the cell cycle and has been shown to reduce the side effects of paclitaxel treatment, such as hematopoietic stem cell death, and to improve its impact on TNBC cells (154). Another study has shown that CDK4/6 inhibitors and paclitaxel synergistically work together by inducing apoptosis in KRAS-mutant lung adenocarcinoma cells (155).

Platinum compounds, another widely used chemotherapeutic agent for cancer treatment, often have side effects such as acute kidney injury (AKI) when applied alone. Kim *et al* (156) demonstrated that CDK4/6 inhibitors can alleviate platinum-associated AKI in an Rb1-dependent manner, but the exact mechanism is unclear. Liu *et al* (157) reported that CDK4/6 inhibitors can inhibit cell proliferation and induce apoptosis to reverse the acquired resistance to platinum compounds in lung cancer cells. The combination of the two in therapy provides a new therapeutic strategy for patients with platinum compound-resistant lung cancer.

Five studies have reported the efficacy of CDK4/6 inhibitors in neoadjuvant therapy, NeoPalAna (158), New MONARCH (74), N007 (159), CORALLEEN (160) and NeoPAL (161). Studies such as NeoPalAna, New MONARCH and N007 mainly focused on the evaluation of the biological effects of CDK4/6 inhibitors combined with endocrine therapy on tumors, such as the reduction of Ki-67. These three studies indicated that CDK4/6 inhibitors such as palbociclib and abemaciclib combined with endocrine therapy can significantly reduce Ki-67 levels and inhibit tumor proliferation, showing a good biological response, and are potentially effective options for neoadjuvant treatment of $HR^+/HER2^-$ BC. Although the treatments evaluated in these studies (74,158,159) were not directly compared with chemotherapy, they laid the groundwork for subsequent comparative trials, such as CORALLEEN (160) and NeoPAL (161), which directly assessed CDK4/6 inhibitors plus endocrine therapy vs. standard chemotherapy. The latter two trials randomly assigned patients to receive neoadjuvant endocrine therapy plus a CDK4/6 inhibitor or neoadjuvant chemotherapy to investigate whether the addition of a CDK4/6 inhibitor to letrozole would provide at least as much clinical benefit as standard chemotherapy. Overall, these two studies showed that in selected patients with $HR^+/HER2^-$ BC, neoadjuvant therapy with a CDK4/6 inhibitor plus letrozole may achieve some equivalent efficacy to conventional chemotherapy, but gaps remain in endpoints such as the rate of pathological complete response (160,161).

Combination ICIs. Increased PD-L1 expression in patients treated with CDK4/6 inhibitors is a clinical problem currently being encountered. It may be one of the potential mechanisms leading to resistance to CDK4/6 inhibitors through evasion of immune-surveillance checkpoints (162).

CDK4/6 inhibitors can be used in combination with ICIs to transform immunologically 'cold' tumors into 'hot' tumors. 'Hot' tumors are more susceptible to immune cell infiltration and are more easy to treat (163). Results from preclinical studies have suggested that ICIs can be a therapeutic strategy to overcome CDK4/6 resistance in BC (164,165). Meanwhile, ICI resistance is associated with cyclin D1 amplification and CDKN2A deletion, which can also be overcome by CDK4/6 inhibition (166-168). Schaer *et al* (56) and Jerby-Arnon *et al* (169) observed synergistic effects exhibited by sequential administration of CDK4/6 inhibitors and ICIs in allogeneic tumor models. Schaer *et al* (56) showed that continuous administration of abemaciclib followed by ICIs, divided into 28 days of treatment, resulted in improved antitumor activity than 14-day treatment or monotherapy. Jerby-Arnon *et al* (169) reported that, as compared with other treatment approaches (vehicle, monotherapy, ICI + abemaciclib Q3D, abemaciclib QD followed by ICI), staged combination therapy (ICIs followed by ICIs plus abemaciclib) resulted in higher rates of tumor suppression and survival in melanoma models.

Consistent with most preclinical data, combination therapy with CDK4/6 inhibitors and ICIs have shown potential in patients with tumors. Rugo *et al* (170), in a phase Ib study of pembrolizumab combined with abemaciclib, found that this combination showed good potential in preliminary clinical efficacy and safety, although further validation is still needed. In an early clinical study involving patients with metastatic disease, Yuan *et al* (171) revealed that palbociclib in combination with pembrolizumab showed a complete remission rate of up to 31% in patients, with median PFS and OS durations of 25.2 and 36.9 months, respectively. Additionally, a previous case report demonstrated that a patient with refractory SMARCA4-deficient small cell carcinoma of the ovary hypercalcemic type experienced disease progression despite multiple treatment regimens, including immunotherapy. Still, the combination of a CDK4/6 inhibitor and ICIs demonstrated efficacy (172). The combination of a CDK4/6 inhibitor and ICIs has also been reported to be effective in treating SMARCA4-deficient cancers and has demonstrated potential (173). Bose *et al* (174) similarly reported a case in which a patient who resisted an ICI alone developed a profound and long-lasting response to combination therapy with a CDK4/6 inhibitor and an ICI.

Because combination therapies with CDK4/6 inhibitors and ICIs have been validated in preclinical studies in overcoming drug resistance and have shown potential in cancer treatment, a number of clinical trials evaluating the safety and efficacy of CDK4/6 inhibitors in combination with ICIs for the treatment of cancer are ongoing (Table SIV) (170,171,175-180). The PACE trial, a multicenter, randomized, open-label phase II trial prospectively evaluated the efficacy of palbociclib in combination with fluvastatin and nivolumab in patients with $ER^+/HER2^-$ MBC after progression following treatment with CDK4/6 inhibitor and AI combination therapy. The results demonstrated that combination therapy with the PD-L1 antibody avelumab and CDK4/6 inhibitors significantly improved PFS (176).

However, combination therapy with CDK4/6 inhibitors and ICIs still has some limitations. Noticeable toxic reactions, especially hematological toxicity such as neutropenia, have become a hindrance limiting the potential of this therapy (171,181,182). Rugo *et al* (170) investigated the safety and efficacy of abemaciclib plus pembrolizumab with/without anastrozole in patients with HR^+/HER^- MBC and reported a spectrum of toxicities with the combination therapy, including an increased incidence and severity of hematological toxicities (such as neutropenia) and immune-related AEs (such as hepatotoxicity and pneumonia). Preliminary reports have indicated that the toxicity-related symptom burden, including but not limited to diarrhea and fatigue, results in a reduction of physical activity scores in the short term. However, these symptoms can be managed effectively, thereby restoring quality of life to a certain extent. Another phase II clinical study reported that the combination of nivolumab and abemaciclib, which has demonstrated greater clinical activity compared with other CDK4/6 inhibitors, resulted in severe and prolonged immune-associated AEs (183). A phase I clinical study by Patnaik *et al* (180) combined LY3300054, a PD-L1 inhibitor, with multiple targeted agents, including abemaciclib. The study reported the impact of hematological toxicity on the long-term prognosis of patients, with some patients discontinuing treatment as a result. Combination therapies with CDK4/6 inhibitors and ICIs currently present both risks and opportunities. If toxicity can be effectively managed, combination therapies have the potential to improve outcomes. However, toxicity management strategies for this combination therapy remain understudied and require further clinical investigation.

It is also worth noting that a previous study (180) revealed that LY3300054 was well tolerated by patients when taken concomitantly with abemaciclib. However, the introduction of abemaciclib prior to combining LY3300054 resulted in hepatotoxicity. These findings suggest that the optimal timing of the combination of CDK4/6 inhibitors and immunotherapy should be explored to maximize efficacy and reduce toxicity. However, to the best of our knowledge, there are currently no studies suggesting the optimal timing of drugs in this combination therapy, and further future clinical trials evaluating this combination therapy are needed to determine the optimal timing and dosage of administration.

Combination targeted therapy. Targeted therapies target specific cancer sites (such as genes or protein molecules in tumor cells), thereby interfering with tumor metastasis, inhibiting tumor cell proliferation and differentiation, and inducing apoptosis of tumor cells (184).

CDK4/6 inhibitors and drugs targeting the PI3K/AKT/mTOR pathway. Extensive preclinical studies have demonstrated that cyclin D1 acts as a common node to achieve crosstalk between CDK4/6 and the PI3K/AKT/mTOR pathway (182). The binding of cyclin D1 to CDK4/6 induces phosphorylation of Rb and its subsequent uncoupling from E2F, which contributes to the progression of the cell cycle in the G_1-S phase (181). Aberrant PI3K/AKT/mTOR pathway activation contributes to CDK4/6 inhibitor resistance (141). Clinical trials have examined the effects of combining PI3K inhibitors and CDK4/6 inhibitors in cancer therapy (Table SV) (185-187). Notably, one trial showed that the triple combination of the PIK3CA-isoform-specific inhibitor alpelisib or the pan-PI3K inhibitor buparlisib with the CDK4/6 inhibitor ribociclib and fulvestrant had high

toxicity (182). Another phase Ib trial tested a triple combination therapy consisting of the CDK4/6 inhibitor palbociclib, the PIK3CA-isoform-specific inhibitor taselisib, and fulvestrant, which showed promising efficacy in $ER^+/HER2^-$ advanced BC with PIK3CA mutations and demonstrated tolerability at pharmacologically active doses (188).

AKT acts as a bridge between the PI3K and mTOR signaling pathways. The TAKTIC study showed that AKT inhibitor therapy in combination with CDK4/6 inhibitors and endocrine therapy was efficacious in some patients and showed good tolerability (189). Table SV includes information three ongoing clinical trials on AKT inhibitors (NCT04862663, NCT04920708 and NCT03959891). Whether AKT inhibitors can be one of the therapeutic options after CDK4/6 inhibitor resistance still awaits validation in clinical trials.

The mTOR pathway is an important signaling pathway downstream of PI3K, and thus, mTOR inhibitors have received as much attention as PI3K inhibitors (190). Everolimus is a typical mTOR inhibitor that has shown promising therapeutic efficacy in the phase III, double-masked, randomized international BOLERO-2 trial (median PFS extension of 4.6 months; $P<0.0001$) (191). For patients resistant to CDK4/6 inhibitors, mTOR inhibitors may provide satisfactory results.

CDK4/6 inhibitors and poly ADP-ribose polymerase (PARP) inhibitors. PARP is a key enzyme involved in the repair of single-stranded DNA gaps. Preclinical studies have shown that the combination of PARP inhibitors, such as olaparib, and CDK4/6 inhibitors can synergistically inhibit the Rb1-E2F1 signaling pathway at both the transcriptional and post-translational levels, leading to cell cycle arrest and downregulation of the E2F1 gene target, ultimately achieving inhibition of tumor cell proliferation, induction of apoptosis and blocking of neuroendocrine differentiation (192-194). Zhu *et al* (195) demonstrated that overexpression of β-catenin, especially hyperphosphorylation of its Ser675 site, activates the Wnt signaling pathway. This process mediates olaparib resistance, which palbociclib significantly attenuates. Currently, several PARP inhibitors, such as olaparib, have been approved by the FDA for cancer treatment, and the potential of combination therapies of PARP inhibitors and CDK4/6 inhibitors in treating cancers such as TNBC has been reported (196).

CDK4/6 inhibitors and other targeted agents. Histone deacetylase (HDAC) inhibitors are also a targeted agent that has received attention. A phase III clinical trial (ACE; NCT02482753) evaluated the efficacy and safety of chidamide combined with exemestane in the treatment of postmenopausal patients with HR^+ advanced BC. The results showed that the modified PFS time was longer with chidamide combined with exemestane (7.4 months) than with placebo combined with exemestane (3.8 months) (197). The San Antonio Breast Cancer Symposium meeting in 2021 reported that entinostat, another HDAC inhibitor, was able to improve the PFS time in patients with $HR^+/HER2^-$ BC (198). HDAC inhibitors may therefore offer new options for combination therapy with CDK4/6 inhibitors and targeted agents.

BCL2 inhibitors are another option for combination therapy with CDK4/6 inhibitors. An early phase I clinical trial demonstrated that venetoclax, a BCL2 inhibitor, was highly tolerated in combination with tamoxifen, and the high level of BCL2 expression in ~70% of patients with ER^+ tumors led to encouraging activity in both ER^+ and $BCL2^+$ metastatic BC (199). However, a phase II clinical trial (Veronica; NCT03584009) showed that venetoclax did not significantly improve PFS in patients who progressed after treatment with CDK4/6 inhibitors (200). There is also an ongoing clinical trial (NCT03900884) designed to evaluate the efficacy, safety and tolerability of a BCL2 inhibitor (venetoclax) in combination with fulvestrant in patients with $ER^+/HER2^-$ MBC. More clinical studies are still needed regarding the therapeutic efficacy of BCL2 inhibitors in combination with endocrine therapy and CDK4/6 inhibitors.

Table SV provides information on combination therapy with CDK4/6 inhibitors and targeted agents. However, combining CDK4/6 inhibitors and targeted agents still has some limitations (201). The optimal timing of including these agents in the therapeutic continuum remains unclear (for example, as part of first-line therapy or at disease progression). As with immunotherapeutic combination therapies, there are still no studies that propose the optimal practical application of drugs in such combination therapies. Further clinical trials evaluating such combination therapies are needed to determine the timing and dosage of administration of the specific therapy in question.

Combination radiation therapy (RT). RT is one of the most widely used local therapeutic options, with palliative RT and ablation commonly used for advanced BC (202). Numerous preclinical studies have shown that CDK4/6 inhibitors can enhance the radiosensitivity of human cancer cell lines by inhibiting the repair mechanism of double DNA scaffold breaks, enhancing apoptosis and blocking cell cycle progression (203-207). This implies that the combination of CDK4/6 inhibitors with RT may increase the antitumor effect of RT but provides a theoretical basis for clinical application.

The NATALEE study (NCT03701334) was a phase III trial that demonstrated the benefit of this combination in patients with stage II and stage III $HR^+/HER2^-$ early BC at risk of recurrence, including patients without lymph node involvement (208). Currently published data on the feasibility of concomitant RT and CDK4/6 inhibitors are based on small-scale retrospective series with low evidence and a lack of prospective clinical data (209). There are currently several ongoing clinical trials evaluating the efficacy of combination therapy with CDK4/6 inhibitors and RT in BC (NCT03691493, NCT03870919, NCT03750396, NCT04563507, NCT05664893 and NCT04923542), almost all of which are in phase I or II. Of these, the prospective phase II ASPIRE trial (NCT03691493) is evaluating RT in combination with palbociclib and hormonal therapy in patients with BC who have developed bone metastases. The PALATINE prospective trial (NCT03870919) will evaluate localized breast RT in combination with CDK4/6 inhibitors in advanced BC.

There is a need to characterize the safety issues of combining CDK4/6 inhibitors and RT, and to help guide therapeutic decisions. A systematic evaluation and meta-analysis has previously been conducted, encompassing 15 studies with a total of 1,133 patients diagnosed with $HR^+/HER2^-$ BC, 617 of whom received CDK4/6 inhibitors in conjunction with RT. The results indicated a 29.4% incidence of severe hematological toxicity, a 2.8% incidence of non-hematological toxicity, a 24.0% CDK4/6 inhibitor dose reduction rate and

a 2.3% discontinuation rate. These findings are analogous to the toxicity rates observed when CDK4/6 inhibitors are used as monotherapy, suggesting that combination therapy does not appear to markedly augment toxicity. However, it is important to note that the majority of the data were derived from retrospective studies, which are subject to certain limitations (202). Another study analyzed 373 patients with MBC who received CDK4/6 inhibitors and RT, and found similar results: The overall toxicity of this combination therapy was shown to be limited, and it may be safe and feasible (210). An observational study revealed that the combination therapy led to an elevated occurrence of grade ≥3 AEs up to 6 weeks following RT. However, it exhibited a higher rate of local control, indicating that the combination therapy achieves a balance between efficacy and toxicity (211). Conversely, a multicenter cohort study observed frequent moderate toxicity reactions to RT with CDK4/6 inhibitors, thereby underscoring the necessity for caution regarding this combination therapy (212).

However, the preclinical rationale suggesting that combined RT and CDK4/6 inhibitors may be unsafe is derived from the cytostatic and immunomodulatory effects reported in studies testing combination therapy in *in vitro* and *in vivo* models (209). Selective inhibition of CDK4/6 may exert its effect on the cell cycle by interfering with the transition from G_1 to S phase, decreasing the level of phosphorylation of Rb proteins, and inducing G_1-phase cell cycle arrest. Irradiated normal cells may exhibit delayed progression through the G_1, S and G_2 phases. Cells demonstrate robust radiation tolerance in the G_0, early G_1 and late S phases of the cell cycle, while exhibiting heightened sensitivity in the late G_1, G_2 and M phases. The combination of CDK4/6 inhibitors and RT has been shown to induce a higher proportion of cells to remain in the G_2/M phase of the cell cycle (1,32,210,213,214).

Preclinical rationale and clinical data on the potential synergistic toxicity of RT and CDK4/6 inhibitor are limited and conflicting, and data on the feasibility are based on low-level evidence from small retrospective series and are somewhat heterogeneous; therefore, caution is recommended in the application of this combination therapy (215).

Potential of combined chimeric antigen receptor (CAR)-T cell therapy. CAR-T cell therapy is a novel and personalized immunotherapeutic approach that has shown promising results in treating hematological malignancies (216). However, the application of this approach in solid tumors has been limited by a variety of factors that may be related to antigenic escape and heterogeneity, limited CAR-T cell transport and infiltration, T-cell depletion and lack of persistence, associated with the immunosuppressive microenvironment in solid tumors (217). Combination therapies may be able to address these limitations. A preclinical study by Lelliot *et al* (218) has shown that inhibition of CDK4/6 activity improves the self-renewal and viability of $CD4^+$ and $CD8^+$ CAR-T memory stem cells, which can enhance the durability and therapeutic efficacy of CAR-T cell therapies. This provides novel insight into the field of CDK4/6 inhibitor combination therapies. This idea is still in preclinical development, and clinical trials combining CDK4/6 inhibitors and CAR-T cell therapy for cancer treatment are lacking.

Safety and tolerability of CDK4/6 inhibitors in combination therapy. With the increasing use of CDK4/6 inhibitors in combination with endocrine therapy, immunotherapy and targeted therapies, clinicians must be aware of associated AEs and their management. Drug-induced AEs often lead to dose reduction and discontinuation, which may affect their efficacy. The safety profile of some of the FDA-approved CDK4/6 inhibitors has been described in the present review.

Nausea, vomiting, fatigue, and cytopenia are common AEs of all CDK4/6 inhibitors (73,79,219,220). Neutropenia has been reported to be a particularly significant AE in the cases of palbociclib and ribociclib. In the PALOMA-2 trial, neutropenia was identified as the most prevalent grade 3/4 AE associated with palbociclib in combination with letrozole for the treatment of advanced BC (59). The MONALEESA-2 study revealed a substantial increase in neutropenia in the treatment of HR^+/HER^- advanced BC with ribociclib in combination with letrozole, with grade 3/4 AEs most frequently characterized by neutropenia (65). The most prevalent AE associated with the administration of abemaciclib was diarrhea, as documented in the MONARCH 2 trial. In this trial, abemaciclib was administered in combination with fulvestrant to patients diagnosed with HR^+/HER^- MBC (68). Additionally, QTc prolongation and hepatobiliary toxicity were identified as significant AEs associated with ribociclib, as reported in the MONALEESA-2 trial (221) of ribociclib in combination with letrozole for the treatment of patients with $HR^+/HER2^-$ MBC, and in the MONALEESA-7 trial (219), respectively. Ribociclib in combination with endocrine therapy has been evaluated in premenopausal women with HR^+ advanced BC. Although the rate of diarrhea was reported to be high among patients receiving abemaciclib, it was usually managed with antidiarrheal medications or dose reductions without discontinuation (222). Abemaciclib has also been reported to be associated with a higher incidence of venous thromboembolism events and a reversible increase in serum creatinine levels (68,71). Hematological AEs are common with CDK4/6 inhibitors, especially in response to combination therapy. CDK6 is particularly important in promoting the proliferation of hematologic precursors, and its inhibition results in decreased production of hematopoietic cells (59,223). Unlike chemotherapy-induced neutropenia, hematological AEs, such as neutropenia, caused by CDK4/6 inhibitors can be rapidly reversed, reflecting cytostatic effects on bone marrow neutrophil precursors (224). Most hematological abnormalities caused by CDK4/6 inhibitors can be adequately managed with standard supportive care and dose modifications (68). Currently, clinical management strategies for the treatment of potential toxicities associated with CDK4/6 inhibitors have been suggested. However, more research is needed to better understand the toxicity profile, and to develop management strategies to minimize drug interruptions and thus optimize the highest treatment effect for patients with BC (223).

7. Discussion

CDK4/6 inhibitors are cell cycle-targeting drugs designed to prevent tumor cell proliferation while restoring normal cell cycle regulation (225). Guidelines have recommended several CDK4/6 inhibitors as first- or second-line drugs for various

tumors. By exploring the clinical application of the aforementioned CDK4/6 inhibitors, it may be concluded that CDK4/6 inhibitors have notable potential in tumor immunotherapy. This is particularly evident when combined with endocrine therapy, chemotherapy, ICIs, targeted therapy, RT and CAR T-cell therapy, as well as other drug combinations, where the combination of related treatments can notably enhance therapeutic efficacy.

However, CDK4/6 inhibitor resistance remains a marked challenge in clinical practice, with several potential resistance mechanisms (138). To address this critical challenge, future research should assess the full scope of the resistance mechanisms mediated by CDK4/6 inhibitors and the potential mechanisms of their synergistic effects with various other therapies (such as endocrine therapy). In addition, treatment options should be sought based on current advances and the resistance mechanisms of CDK4/6 inhibitors in combination with endocrine therapy should be determined through appropriate clinical validation.

Protein degraders can increase selectivity, improve efficacy and overcome drug resistance; they may also provide a solution to the problem of CDK4/6 inhibitor resistance, which limits the use of these inhibitors (226). The degradation products of CDK can induce the ubiquitination and proteasomal degradation of CDK, thereby reducing the expression levels and stability of CDKs and achieving the purpose of CDK inhibition. Currently, the results of preclinical studies have shown that in $ER^+/HER2^-$ BC models, compared with clinically approved CDK4/6 inhibitors, CDK4/6 degraders exhibit excellent single-agent antitumor activity both *in vitro* and *in vivo* (227). However, clinical trials using CDK4/6 degraders to overcome CDK4/6 inhibitor resistance are at an early stage. ARV-471 is a selective, orally available proteolysis-targeting chimera protein degrader targeting wild-type and mutant ER. In the phase I/II VERITAC trial (NCT04072952), ARV-471 demonstrated clinical activity in patients with advanced $HR^+/HER2^-$ BC who had received prior treatment with a CDK4/6 inhibitor (228). In addition, the phase III VERITAC-2 trial (NCT05654623) is ongoing to compare the efficacy of ARV-471 with fulvestrant in patients who have failed treatment with a CDK4/6 inhibitor (229). CDK4/6 degraders show potential clinical value in overcoming drug resistance, but their clinical application still requires more research and trials to verify their safety and efficacy.

The development and testing of novel CDK4/6 inhibitors are also key research directions in the future. Given that the activation of CDK2 represents a common mechanism of resistance to CDK4/6 inhibitors, the development of compounds that inhibit CDK4/6 and CDK2 may prevent or delay the occurrence of resistance (13). Furthermore, a novel CDK4/6 inhibitor compound with lower alkalinity may evade lysosome isolation and be effective against drug-resistant cancer types, such as TNBC (230). Novel CDK inhibitor compounds from different synthetic frameworks are currently under investigation, examples of such derivatives include amidoyl, pyrazole, quinazoline and pyrimidine derivatives (231). In the forthcoming years, we may observe the advent of novel CDK4/6 inhibitors that have exhibited substantial promise in preclinical studies and clinical trials.

In addition, CDK4/6 inhibitors possess inherent limitations in clinical practice. The most notable challenge confronting these novel targeted therapies is the absence of predictive molecular biomarkers. The clinical efficacy of CDK4/6 inhibitors in treatment is contingent upon developing predictive biomarkers and biologically reasonable combination therapies (232). In actual clinical settings, the AEs caused by CDK4/6 inhibitors are also worth consideration. Although there are currently clinical management recommendations regarding the potential toxicity of CDK4/6 inhibitors, further research is still needed to understand their toxicological characteristics and to continuously optimize management strategies comprehensively. This will contribute to reducing the occurrence of treatment interruptions and improving the treatment efficacy and compliance of patients with BC (223).

8. Conclusion

The present study reviewed the regulatory role of CDK4/6 inhibitors in tumor immunity and the potential value of tumor immunotherapy. CDK4/6 inhibitors affect tumors by regulating the cell cycle, changing the immune microenvironment and regulating immune checkpoints, and they have garnered attention as clinical drugs for the treatment of various types of cancer. Du *et al* (233) thoroughly explored their clinical application in malignant solid tumors, alongside strategies for combining these inhibitors with endocrine or targeted therapies. Sun *et al* (234) delved into the immunomodulatory effects of targeting CDK4/6 in cancer, whereas Glaviano *et al* (138) offered an in-depth analysis of resistance mechanisms associated with CDK4/6 inhibitors. By contrast, the present review provides a more systematic account of recent advances in CDK4/6 inhibitors in tumor immunity and resistance mechanisms, with a comprehensive review of recent advances in clinical trials. This integrated perspective may reflect current research dynamics more comprehensively than the existing literature, providing researchers with a unified frame of reference.

In response to the problem of patient resistance to CDK4/6 inhibitors, the current review further proposed a variety of combination treatment strategies to enhance drug sensitivity and improve efficacy. This direction has been less explored in the existing literature and clinical trials. Although CDK4/6 inhibitors show promising applications, the review concludes with an objective analysis of current challenges and a clear direction for future development, such as identifying predictive molecular biomarkers and addressing safety issues, which need to be validated in more clinical trials.

In summary, the present study systematically reviewed the latest progress of CDK4/6 inhibitors in the field of tumor immunity, proposed an innovative combination therapy strategy and provided a clear direction for future research, showing its unique contribution. These innovations not only provide novel ideas for overcoming drug resistance and improving the effects of tumor immunotherapy, but also provide suggestions for future follow-up research and verification.

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Authors' contributions

JX and YW drafted and revised the manuscript. FH, QZ, YC, SG, YX and RS collected the relevant papers and helped to revise the manuscript. FH, QZ and YC designed the tables and charts. JX and YW reviewed the article. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no known competing interests.

References

1. Xu H, Yu S, Liu Q, Yuan X, Mani S, Pestell RG and Wu K: Recent advances of highly selective CDK4/6 inhibitors in breast cancer. J Hematol Oncol 10: 97, 2017.
2. Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW and Jager A: Inhibiting CDK4/6 in breast cancer with palbociclib, ribociclib, and abemaciclib: Similarities and differences. Drugs 81: 317-331, 2021.
3. CDK4/6 inhibitors induce antitumor immunity. Cancer Discov 7: 1052, 2017.
4. Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, Khan N, Ubellacker JM, Xie S, Metzger-Filho O, *et al*: CDK4/6 inhibition triggers anti-tumor immunity. Nature 548: 471-475, 2017.
5. Kong T, Xue Y, Cencic R, Zhu X, Monast A, Fu Z, Pilon V, Sangwan V, Guiot MC, Foulkes WD, *et al*: eIF4A inhibitors suppress cell-cycle feedback response and acquired resistance to CDK4/6 inhibition in cancer. Mol Cancer Ther 18: 2158-2170, 2019.
6. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, Ettl J, Patel R, Pinter T, Schmidt M, *et al*: The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): A randomised phase 2 study. Lancet Oncol 16: 25-35, 2015.
7. Lei ZN, Tian Q, Teng QX, Wurpel JND, Zeng L, Pan Y and Chen ZS: Understanding and targeting resistance mechanisms in cancer. MedComm (2020) 4: e265, 2023.
8. Verma S, Bartlett CH, Schnell P, DeMichele AM, Loi S, Ro J, Colleoni M, Iwata H, Harbeck N, Cristofanilli M, *et al*: Palbociclib in combination with fulvestrant in women with hormone receptor-Positive/HER2-Negative advanced metastatic breast cancer: Detailed safety analysis from a multicenter, randomized, Placebo-controlled, phase III study (PALOMA-3). Oncologist 21: 1165-1175, 2016.
9. Yang Y, Luo J, Chen X, Yang Z, Mei X, Ma J, Zhang Z, Guo X and Yu X: CDK4/6 inhibitors: A novel strategy for tumor radiosensitization. J Exp Clin Cancer Res 39: 188, 2020.
10. Sobhani N, D'Angelo A, Pittacolo M, Roviello G, Miccoli A, Corona SP, Bernocchi O, Generali D and Otto T: Updates on the CDK4/6 inhibitory strategy and combinations in breast cancer. Cells 8: 321, 2019.
11. Abraham J, Coleman R, Elias A, Holmes FA, Kalinsky K, Kittaneh M, Lower E, Mahtani R, Terry Mamounas E, Pegram M, *et al*: Use of cyclin-dependent kinase (CDK) 4/6 inhibitors for hormone receptor-positive, human epidermal growth factor receptor 2-negative, metastatic breast cancer: A roundtable discussion by The Breast Cancer Therapy Expert Group (BCTEG). Breast Cancer Res Treat 171: 11-20, 2018.
12. Johnson J, Thijssen B, McDermott U, Garnett M, Wessels LFA and Bernards R: Targeting the RB-E2F pathway in breast cancer. Oncogene 35: 4829-4835, 2016.
13. Fassl A, Geng Y and Sicinski P: CDK4 and CDK6 kinases: From basic science to cancer therapy. Science 375: eabc1495, 2022.
14. Hallett ST, Pastok MW, Morgan RML, Wittner A, Blundell KLIM, Felletar I, Wedge SR, Prodromou C, Noble MEM, Pearl LH and Endicott JA: Differential regulation of G1 CDK complexes by the Hsp90-Cdc37 chaperone system. Cell Rep 21: 1386-1398, 2017.
15. Kwapisz D: Cyclin-dependent kinase 4/6 inhibitors in breast cancer: Palbociclib, ribociclib, and abemaciclib. Breast Cancer Res Treat 166: 41-54, 2017.
16. Guo L, Qi J, Wang H, Jiang X and Liu Y: Getting under the skin: The role of CDK4/6 in melanomas. Eur J Med Chem 204: 112531, 2020.
17. Pan Q, Luo P, Hu K, Qiu Y, Liu G, Dai S, Cui B, Yin D and Shi C: Periodic changes of cyclin D1 mRNA stability are regulated by PC4 modifications in the cell cycle. J Cell Biol 223: e202308066, 2024.
18. O'Leary B, Finn RS and Turner NC: Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol 13: 417-430, 2016.
19. Cai Z, Shi Q, Li Y, Jin L, Li S, Wong LL, Wang J, Jiang X, Zhu M, Lin J, *et al*: LncRNA EILA promotes CDK4/6 inhibitor resistance in breast cancer by stabilizing cyclin E1 protein. Sci Adv 9: eadi3821, 2023.
20. Beleut M, Rajaram RD, Caikovski M, Ayyanan A, Germano D, Choi Y, Schneider P and Brisken C: Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. Proc Natl Acad Sci USA 107: 2989-2994, 2010.
21. Błaszczak-Świątkiewicz K: New selective progesterone receptor modulators and their impact on the RANK/RANKL complex activity. Molecules 25: 1321, 2020.
22. Mauro L, Pellegrino M, Giordano F, Ricchio E, Rizza P, De Amicis F, Catalano S, Bonofiglio D, Panno ML and Andò S: Estrogen receptor-α drives adiponectin effects on cyclin D1 expression in breast cancer cells. FASEB J 29: 2150-2160, 2015.
23. Yang C, Chen L, Li C, Lynch MC, Brisken C and Schmidt EV: Cyclin D1 enhances the response to estrogen and progesterone by regulating progesterone receptor expression. Mol Cell Biol 30: 3111-3125, 2010.
24. Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, Pearson A, Guzman M, Rodriguez O, Grueso J, *et al*: Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. Cancer Res 76: 2301-2313, 2016.
25. Huang X, You L, Nepovimova E, Psotka M, Malinak D, Valko M, Sivak L, Korabecny J, Heger Z, Adam V, *et al*: Inhibitors of phosphoinositide 3-kinase (PI3K) and phosphoinositide 3-kinase-related protein kinase family (PIKK). J Enzyme Inhib Med Chem 38: 2237209, 2023.
26. Presti D and Quaquarini E: The PI3K/AKT/mTOR and CDK4/6 pathways in endocrine resistant HR+/HER2-metastatic breast cancer: Biological mechanisms and new treatments. Cancers (Basel) 11: 1242, 2019.
27. Hao C, Wei Y, Meng W, Zhang J and Yang X: PI3K/AKT/mTOR inhibitors for hormone receptor-positive advanced breast cancer. Cancer Treat Rev 132: 102861, 2025.
28. Liu Q, Liu Y, Li X, Wang D, Zhang A, Pang J, He J, Chen X and Tang NJ: Perfluoroalkyl substances promote breast cancer progression via ERα and GPER mediated PI3K/Akt and MAPK/Erk signaling pathways. Ecotoxicol Environ Saf 258: 114980, 2023.
29. Luboff AJ and DeRemer DL: Capivasertib: A novel AKT inhibitor approved for hormone-receptor-positive, HER-2-negative metastatic breast cancer. Ann Pharmacother 58: 1229-1237, 2024.

30. Michaloglou C, Crafter C, Siersbaek R, Delpuech O, Curwen JO, Carnevalli LS, Staniszewska AD, Polanska UM, Cheraghchi-Bashi A, Lawson M, *et al*: Combined inhibition of mTOR and CDK4/6 is required for optimal blockade of E2F function and long-term growth inhibition in estrogen receptor-positive breast cancer. Mol Cancer Ther 17: 908-920, 2018.
31. Teh JLF and Aplin AE: Arrested developments: CDK4/6 inhibitor resistance and alterations in the tumor immune microenvironment. Clin Cancer Res 25: 921-927, 2019.
32. Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, Chhabra S, Huang W, Liu H, Aref AR, *et al*: CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. Cancer Discov 8: 216-233, 2018.
33. Mognol GP, Carneiro FRG, Robbs BK, Faget DV and Viola JPB: Cell cycle and apoptosis regulation by NFAT transcription factors: New roles for an old player. Cell Death Dis 7: e2199, 2016.
34. Liu J, Cheng M, Xu J, Liang Y, Yin B and Liang J: Effect of CDK4/6 inhibitors on tumor immune microenvironment. Immunol Invest 53: 437-449, 2024.
35. Scirocchi F, Scagnoli S, Botticelli A, Di Filippo A, Napoletano C, Zizzari IG, Strigari L, Tomao S, Cortesi E, Rughetti A, *et al*: Immune effects of CDK4/6 inhibitors in patients with HR+/HER2-metastatic breast cancer: Relief from immunosuppression is associated with clinical response. EBioMedicine 79: 104010, 2022.
36. Kent LN and Leone G: The broken cycle: E2F dysfunction in cancer. Nat Rev Cancer 19: 326-338, 2019.
37. Kohlmeyer JL, Lingo JJ, Kaemmer CA, Scherer A, Warrier A, Voigt E, Raygoza Garay JA, McGivney GR, Brockman QR, Tang A, *et al*: CDK4/6-MEK inhibition in MPNSTs causes plasma cell infiltration, sensitization to PD-L1 blockade, and tumor regression. Clin Cancer Res 29: 3484-3497, 2023.
38. Ruscetti M, Leibold J, Bott MJ, Fennell M, Kulick A, Salgado NR, Chen CC, Ho YJ, Sanchez-Rivera FJ, Feucht J, *et al*: NK cell-mediated cytotoxicity contributes to tumor control by a cytostatic drug combination. Science 362: 1416-1422, 2018.
39. Llanos S, Megias D, Blanco-Aparicio C, Hernández-Encinas E, Rovira M, Pietrocola F and Serrano M: Lysosomal trapping of palbociclib and its functional implications. Oncogene 38: 3886-3902, 2019.
40. De Buck M, Gouwy M, Wang JM, Van Snick J, Opdenakker G, Struyf S and Van Damme J: Structure and expression of different serum amyloid A (SAA) variants and their concentration-dependent functions during host insults. Curr Med Chem 23: 1725-1755, 2016.
41. Sagiv A, Biran A, Yon M, Simon J, Lowe SW and Krizhanovsky V: Granule exocytosis mediates immune surveillance of senescent cells. Oncogene 32: 1971-1977, 2013.
42. Ritschka B, Storer M, Mas A, Heinzmann F, Ortells MC, Morton JP, Sansom OJ, Zender L and Keyes WM: The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. Genes Dev 31: 172-183, 2017.
43. Muñoz-Espín D and Serrano M: Cellular senescence: From physiology to pathology. Nat Rev Mol Cell Biol 15: 482-496, 2014.
44. Favaretto G, Rossi MN, Cuollo L, Laffranchi M, Cervelli M, Soriani A, Sozzani S, Santoni A and Antonangeli F: Neutrophil-activating secretome characterizes palbociclib-induced senescence of breast cancer cells. Cancer Immunol Immunother 73: 113, 2024.
45. Mehta AK, Cheney EM, Hartl CA, Pantelidou C, Oliwa M, Castrillon JA, Lin JR, Hurst KE, de Oliveira Taveira M, Johnson NT, *et al*: Targeting immunosuppressive macrophages overcomes PARP inhibitor resistance in BRCA1-associated triple-negative breast cancer. Nat Cancer 2: 66-82, 2021.
46. Gu M, Liu Y, Zheng W, Jing Z, Li X, Guo W, Zhao Z, Yang X, Liu Z, Zhu X and Gao W: Combined targeting of senescent cells and senescent macrophages: A new idea for integrated treatment of lung cancer. Semin Cancer Biol 106-107: 43-57, 2024.
47. Fang Y, Zhang Z, Liu Y, Gao T, Liang S, Chu Q, Guan L, Mu W, Fu S, Yang H, *et al*: Artificial assembled macrophage Co-deliver black phosphorus quantum dot and CDK4/6 inhibitor for colorectal cancer triple-therapy. ACS Appl Mater Interfaces 14: 20628-20640, 2022.
48. Kumar A, Ramani V, Bharti V, de Lima Bellan D, Saleh N, Uzhachenko R, Shen C, Arteaga C, Richmond A, Reddy SM and Vilgelm A: Dendritic cell therapy augments antitumor immunity triggered by CDK4/6 inhibition and immune checkpoint blockade by unleashing systemic CD4 T-cell responses. J Immunother Cancer 11: e006019, 2023.
49. Zhang J, Bu X, Wang H, Zhu Y, Geng Y, Nihira NT, Tan Y, Ci Y, Wu F, Dai X, *et al*: Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. Nature 553: 91-95, 2018.
50. Filippone A, Lanza M, Mannino D, Raciti G, Colarossi C, Sciacca D, Cuzzocrea S and Paterniti I: PD1/PD-L1 immune checkpoint as a potential target for preventing brain tumor progression. Cancer Immunol Immunother 71: 2067-2075, 2022.
51. Hossain MA, Liu G, Dai B, Si Y, Yang Q, Wazir J, Birnbaumer L and Yang Y: Reinvigorating exhausted $CD8^+$ cytotoxic T lymphocytes in the tumor microenvironment and current strategies in cancer immunotherapy. Med Res Rev 41: 156-201, 2021.
52. Szeto C, Lobos CA, Nguyen AT and Gras S: TCR recognition of peptide-MHC-I: Rule makers and breakers. Int J Mol Sci 22: 68, 2020.
53. Wu X, Gu Z, Chen Y, Chen B, Chen W, Weng L and Liu X: Application of PD-1 blockade in cancer immunotherapy. Comput Struct Biotechnol J 17: 661-674, 2019.
54. Wu M, Huang Q, Xie Y, Wu X, Ma H, Zhang Y and Xia Y: Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation. J Hematol Oncol 15: 24, 2022.
55. Seto T, Sam D and Pan M: Mechanisms of primary and secondary resistance to immune checkpoint inhibitors in cancer. Med Sci (Basel) 7: 14, 2019.
56. Schaer DA, Beckmann RP, Dempsey JA, Huber L, Forest A, Amaladas N, Li Y, Wang YC, Rasmussen ER, Chin D, *et al*: The CDK4/6 inhibitor abemaciclib induces a T cell inflamed tumor microenvironment and enhances the efficacy of PD-L1 checkpoint blockade. Cell Rep 22: 2978-2994, 2018.
57. Shrestha M, Wang DY, Ben-David Y and Zacksenhaus E: CDK4/6 inhibitors and the pRB-E2F1 axis suppress PVR and PD-L1 expression in triple-negative breast cancer. Oncogenesis 12: 29, 2023.
58. Beaver JA, Amiri-Kordestani L, Charlab R, Chen W, Palmby T, Tilley A, Zirkelbach JF, Yu J, Liu Q, Zhao L, *et al*: FDA Approval: Palbociclib for the treatment of postmenopausal patients with estrogen Receptor-positive, HER2-Negative metastatic breast cancer. Clin Cancer Res 21: 4760-4766, 2015.
59. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, Harbeck N, Lipatov ON, Walshe JM, Moulder S, *et al*: Palbociclib and letrozole in advanced breast cancer. N Engl J Med 375: 1925-1936, 2016.
60. Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, *et al*: Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): Final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 17: 425-439, 2016.
61. Wedam S, Fashoyin-Aje L, Bloomquist E, Tang S, Sridhara R, Goldberg KB, Theoret MR, Amiri-Kordestani L, Pazdur R and Beaver JA: FDA approval summary: Palbociclib for male patients with metastatic breast cancer. Clin Cancer Res 26: 1208-1212, 2020.
62. Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ, Ginther C, Atefi M, Chen I, Fowst C, *et al*: PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 11: R77, 2009.
63. Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im SA, Petrakova K, Bianchi GV, Esteva FJ, Martín M, *et al*: Overall survival with ribociclib plus fulvestrant in advanced breast cancer. N Engl J Med 382: 514-524, 2020.
64. Im SA, Lu YS, Bardia A, Harbeck N, Colleoni M, Franke F, Chow L, Sohn J, Lee KS, Campos-Gomez S, *et al*: Overall survival with ribociclib plus endocrine therapy in breast cancer. N Engl J Med 381: 307-316, 2019.
65. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, Campone M, Blackwell KL, André F, Winer EP, *et al*: Ribociclib as First-line therapy for HR-positive, advanced breast cancer. N Engl J Med 375: 1738-1748, 2016.
66. Research C for DE and: FDA expands ribociclib indication in HR-positive, HER2-negative advanced or metastatic breast cancer. FDA, 2024.

67. Hortobagyi GN, Lacko A, Sohn J, Cruz F, Ruiz Borrego M, Manikhas A, Hee Park Y, Stroyakovskiy D, Yardley DA, Huang CS, *et al*: A phase III trial of adjuvant ribociclib plus endocrine therapy versus endocrine therapy alone in patients with HR-positive/HER2-negative early breast cancer: Final invasive disease-free survival results from the NATALEE trial. Ann Oncol 36: 149-157, 2025.
68. Sledge GW, Toi M, Neven P, Sohn J, Inoue K, Pivot X, Burdaeva O, Okera M, Masuda N, Kaufman PA, *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 35: 2875-2884, 2017.
69. Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, Park IH, Trédan O, Chen SC, Manso L, *et al*: MONARCH 3: Abemaciclib as initial therapy for advanced breast cancer. J Clin Oncol 35: 3638-3646, 2017.
70. Dickler MN, Tolaney SM, Rugo HS, Cortés J, Diéras V, Patt D, Wildiers H, Hudis CA, O'Shaughnessy J, Zamora E, *et al*: MONARCH 1, A Phase II study of abemaciclib, a CDK4 and CDK6 inhibitor, as a single agent, in patients with refractory HR+/HER2-Metastatic breast cancer. Clin Cancer Res 23: 5218-5224, 2017.
71. Johnston SRD, Harbeck N, Hegg R, Toi M, Martin M, Shao ZM, Zhang QY, Martinez Rodriguez JL, Campone M, Hamilton E, *et al*: Abemaciclib combined with endocrine therapy for the adjuvant treatment of HR+, HER2-, Node-positive, High-risk, early breast cancer (monarchE). J Clin Oncol 38: 3987-3998, 2020.
72. Harbeck N, Rastogi P, Martin M, Tolaney SM, Shao ZM, Fasching PA, Huang CS, Jaliffe GG, Tryakin A, Goetz MP, *et al*: Adjuvant abemaciclib combined with endocrine therapy for high-risk early breast cancer: Updated efficacy and Ki-67 analysis from the monarchE study. Ann Oncol 32: 1571-1581, 2021.
73. Royce M, Mulkey F, Osgood C, Bloomquist E and Amiri-Kordestani L: US food and drug administration expanded adjuvant indication of abemaciclib in High-risk early breast cancer. J Clin Oncol 41: 3456-3457, 2023.
74. Sledge GW, Toi M, Neven P, Sohn J, Inoue K, Pivot X, Burdaeva O, Okera M, Masuda N, Kaufman PA, *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 6: 116-124, 2020.
75. Turner NC, Slamon DJ, Ro J, Bondarenko I, Im SA, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, *et al*: Overall survival with palbociclib and fulvestrant in advanced breast cancer. N Engl J Med 379: 1926-1936, 2018.
76. Desnoyers A, Nadler MB, Kumar V, Saleh R and Amir E: Comparison of treatment-related adverse events of different Cyclin-dependent kinase 4/6 inhibitors in metastatic breast cancer: A network meta-analysis. Cancer Treat Rev 90: 102086, 2020.
77. Kappel C, Elliott MJ, Kumar V, Nadler MB, Desnoyers A and Amir E: Comparative overall survival of CDK4/6 inhibitors in combination with endocrine therapy in advanced breast cancer. Sci Rep 14: 3129, 2024.
78. Slamon D, Lipatov O, Nowecki Z, McAndrew N, Kukielka-Budny B, Stroyakovskiy D, Yardley DA, Huang CS, Fasching PA, Crown J, *et al*: Ribociclib plus endocrine therapy in early breast cancer. N Engl J Med 390: 1080-1091, 2024.
79. Johnston S, Martin M, Di Leo A, Im SA, Awada A, Forrester T, Frenzel M, Hardebeck MC, Cox J, Barriga S, *et al*: MONARCH 3 final PFS: A randomized study of abemaciclib as initial therapy for advanced breast cancer. NPJ Breast Cancer 5: 5, 2019.
80. Carlino L, Christodoulou MS, Restelli V, Caporuscio F, Foschi F, Semrau MS, Costanzi E, Tinivella A, Pinzi L, Lo Presti L, *et al*: Structure-activity relationships of hexahydrocyclopenta[c]quinoline derivatives as allosteric inhibitors of CDK2 and EGFR. ChemMedChem 13: 2627-2634, 2018.
81. Bisi JE, Sorrentino JA, Jordan JL, Darr DD, Roberts PJ, Tavares FX and Strum JC: Preclinical development of G1T38: A novel, potent and selective inhibitor of cyclin dependent kinases 4/6 for use as an oral antineoplastic in patients with CDK4/6 sensitive tumors. Oncotarget 8: 42343-42358, 2017.
82. Profitós-Pelejà N, Ribeiro ML, Parra J, Fernández-Serrano M, Marin-Escudero P, Makovski-Silverstein A, Cosenza S, Esteller M and Roué G: Prolonged cell cycle arrest by the CDK4/6 antagonist narazaciclib restores ibrutinib response in preclinical models of BTKi-resistant mantle cell lymphoma. Hematological Oncol 41: 553-554, 2023.
83. Freeman-Cook KD, Hoffman RL, Behenna DC, Boras B, Carelli J, Diehl W, Ferre RA, He YA, Hui A, Huang B, *et al*: Discovery of PF-06873600, a CDK2/4/6 inhibitor for the treatment of cancer. J Med Chem 64: 9056-9077, 2021.
84. Liu C, Liu B, Xu C, Zhang P and Yu C: ETH-155008, a novel selective dual inhibitor of FLT3 and CDK4/6 in preclinical treatment of acute myeloid leukemia. Blood 134: 5141-5141, 2019.
85. Gelbert LM, Cai S, Lin X, Sanchez-Martinez C, Del Prado M, Lallena MJ, Torres R, Ajamie RT, Wishart GN, Flack RS, *et al*: Preclinical characterization of the CDK4/6 inhibitor LY2835219: In-vivo cell cycle-dependent/independent anti-tumor activities alone/in combination with gemcitabine. Invest New Drugs 32: 825-837, 2014.
86. Goel S, Tan AR, Rugo HS, Aftimos P, Andrić Z, Beelen A, Zhang J, Yi JS, Malik R and O'Shaughnessy J: Trilaciclib prior to gemcitabine plus carboplatin for metastatic triple-negative breast cancer: Phase III PRESERVE 2. Future Oncol 18: 3701-3711, 2022.
87. Lenz HJ, Liu T, Chen EY, Horváth Z, Bondarenko I, Danielewicz I, Ghidini M, García-Alfonso P, Jones R, Aapro M, *et al*: Trilaciclib prior to FOLFOXIRI/bevacizumab for patients with untreated metastatic colorectal cancer: Phase 3 PRESERVE 1 trial. JNCI Cancer Spectr 9: pkae116, 2025.
88. Zhang J, Yang N, Ji D, Shen W, Li W, Han R, Wang N, Tao H, Chapman SC, Sykes AK, *et al*: A Randomized phase i study of abemaciclib in chinese patients with Advanced and/or metastatic cancers. Target Oncol 16: 177-187, 2021.
89. DeWire M, Fuller C, Hummel TR, Chow LML, Salloum R, de Blank P, Pater L, Lawson S, Zhu X, Dexheimer P, *et al*: A phase I/II study of ribociclib following radiation therapy in children with newly diagnosed diffuse intrinsic pontine glioma (DIPG). J Neurooncol 149: 511-522, 2020.
90. Hart LL, Ferrarotto R, Andric ZG, Beck JT, Subramanian J, Radosavljevic DZ, Zaric B, Hanna WT, Aljumaily R, Owonikoko TK, *et al*: Myelopreservation with trilaciclib in patients receiving topotecan for small cell lung cancer: Results from a randomized, double-blind, placebo-controlled phase II study. Adv Ther 38: 350-365, 2021.
91. Xu B, Li H, Zhang Q, Sun W, Yu Y, Li W, Wang S, Liao N, Shen P, Liu Y, *et al*: Pharmacokinetics, safety, activity, and biomarker analysis of palbociclib plus letrozole as first-line treatment for ER+/HER2-advanced breast cancer in Chinese women. Cancer Chemother Pharmacol 88: 131-141, 2021.
92. Miller TW, Traphagen NA, Li J, Lewis LD, Lopes B, Asthagiri A, Loomba J, De Jong J, Schiff D, Patel SH, *et al*: Tumor pharmacokinetics and pharmacodynamics of the CDK4/6 inhibitor ribociclib in patients with recurrent glioblastoma. J Neurooncol 144: 563-572, 2019.
93. Wander SA, Cohen O, Gong X, Johnson GN, Buendia-Buendia JE, Lloyd MR, Kim D, Luo F, Mao P, Helvie K, *et al*: The genomic landscape of intrinsic and acquired resistance to Cyclin-dependent kinase 4/6 inhibitors in patients with hormone Receptor-positive metastatic breast cancer. Cancer Discov 10: 1174-1193, 2020.
94. Kim S, Leong A, Kim M and Yang HW: CDK4/6 initiates Rb inactivation and CDK2 activity coordinates cell-cycle commitment and G1/S transition. Sci Rep 12: 16810, 2022.
95. Fisher RP: Getting to S: CDK functions and targets on the path to cell-cycle commitment. F1000Res 5: 2374, 2016.
96. Kim S, Armand J, Safonov A, Zhang M, Soni RK, Schwartz G, McGuinness JE, Hibshoosh H, Razavi P, Kim M, *et al*: Sequential activation of E2F via Rb degradation and c-Myc drives resistance to CDK4/6 inhibitors in breast cancer. Cell Rep 42: 113198, 2023.
97. O'Leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, André F, Loibl S, Loi S, Garcia-Murillas I, *et al*: The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 8: 1390-1403, 2018.
98. Sharma A, Comstock CES, Knudsen ES, Cao KH, Hess-Wilson JK, Morey LM, Barrera J and Knudsen KE: Retinoblastoma tumor suppressor status is a critical determinant of therapeutic response in prostate cancer cells. Cancer Res 67: 6192-6203, 2007.
99. Malumbres M, Sotillo R, Santamaría D, Galán J, Cerezo A, Ortega S, Dubus P and Barbacid M: Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell 118: 493-504, 2004.
100. Pandey K, An HJ, Kim SK, Lee SA, Kim S, Lim SM, Kim GM, Sohn J and Moon YW: Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: A review. Int J Cancer 145: 1179-1188, 2019.

101. Goel S, Bergholz JS and Zhao JJ: Targeting CDK4 and CDK6 in cancer. Nat Rev Cancer 22: 356-372, 2022.
102. Cen L, Carlson BL, Schroeder MA, Ostrem JL, Kitange GJ, Mladek AC, Fink SR, Decker PA, Wu W, Kim JS, *et al*: p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells. Neuro Oncol 14: 870-881, 2012.
103. Iwata S, Tatsumi Y, Yonemoto T, Araki A, Itami M, Kamoda H, Tsukanishi T, Hagiwara Y, Kinoshita H, Ishii T, *et al*: CDK4 overexpression is a predictive biomarker for resistance to conventional chemotherapy in patients with osteosarcoma. Oncol Rep 46: 135, 2021.
104. Schachter MM, Merrick KA, Larochelle S, Hirschi A, Zhang C, Shokat KM, Rubin SM and Fisher RP: A Cdk7-Cdk4 T-loop phosphorylation cascade promotes G1 progression. Mol Cell 50: 250-260, 2013.
105. Coombes RC, Howell S, Lord SR, Kenny L, Mansi J, Mitri Z, Palmieri C, Chap LI, Richards P, Gradishar W, *et al*: Dose escalation and expansion cohorts in patients with advanced breast cancer in a Phase I study of the CDK7-inhibitor samuraciclib. Nat Commun 14: 4444, 2023.
106. Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, Baselga J, Rosen N and Chandarlapaty S: Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene 36: 2255-2264, 2017.
107. Schoninger SF and Blain SW: The ongoing search for biomarkers of CDK4/6 inhibitor responsiveness in breast cancer. Mol Cancer Ther 19: 3-12, 2020.
108. Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, Hsieh W, Sanchez-Vega F, Brown DN, Da Cruz Paula AF, *et al*: Loss of the FAT1 tumor suppressor promotes resistance to CDK4/6 inhibitors via the hippo pathway. Cancer Cell 34: 893-905.e8, 2018.
109. Cornell L, Wander SA, Visal T, Wagle N and Shapiro GI: MicroRNA-mediated suppression of the TGF-β pathway confers transmissible and reversible CDK4/6 inhibitor resistance. Cell Rep 26: 2667-2680.e7, 2019.
110. Chu C, Geng Y, Zhou Y and Sicinski P: Cyclin E in normal physiology and disease states. Trends Cell Biol 31: 732-746, 2021.
111. Watt AC and Goel S: Cellular mechanisms underlying response and resistance to CDK4/6 inhibitors in the treatment of hormone receptor-positive breast cancer. Breast Cancer Res 24: 17, 2022.
112. Chandarlapaty S and Razavi P: Cyclin E mRNA: Assessing Cyclin-dependent kinase (CDK) activation state to elucidate breast cancer resistance to CDK4/6 Inhibitors. J Clin Oncol 37: 1148-1150, 2019.
113. Guarducci C, Bonechi M, Benelli M, Biagioni C, Boccalini G, Romagnoli D, Verardo R, Schiff R, Osborne CK, De Angelis C, *et al*: Cyclin E1 and Rb modulation as common events at time of resistance to palbociclib in hormone receptor-positive breast cancer. NPJ Breast Cancer 4: 38, 2018.
114. Turner NC, Liu Y, Zhu Z, Loi S, Colleoni M, Loibl S, DeMichele A, Harbeck N, André F, Bayar MA, *et al*: Cyclin E1 expression and palbociclib efficacy in previously treated hormone Receptor-positive metastatic breast cancer. J Clin Oncol 37: 1169-1178, 2019.
115. Al-Qasem AJ, Alves CL, Ehmsen S, Tuttolomondo M, Terp MG, Johansen LE, Vever H, Hoeg LVA, Elias D, Bak M and Ditzel HJ: Co-targeting CDK2 and CDK4/6 overcomes resistance to aromatase and CDK4/6 inhibitors in ER+ breast cancer. NPJ Precis Oncol 6: 68, 2022.
116. Gong X, Du J, Parsons SH, Merzoug FF, Webster Y, Iversen PW, Chio LC, Van Horn RD, Lin X, Blosser W, *et al*: Aurora a kinase inhibition is synthetic lethal with loss of the RB1 tumor suppressor gene. Cancer Discov 9: 248-263, 2019.
117. Hsueh KW, Fu SL, Chang CB, Chang YL and Lin CH: A novel Aurora-A-mediated phosphorylation of p53 inhibits its interaction with MDM2. Biochim Biophys Acta 1834: 508-515, 2013.
118. Turner N and Grose R: Fibroblast growth factor signalling: From development to cancer. Nat Rev Cancer 10: 116-129, 2010.
119. Formisano L, Lu Y, Servetto A, Hanker AB, Jansen VM, Bauer JA, Sudhan DR, Guerrero-Zotano AL, Croessmann S, Guo Y, *et al*: Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. Nat Commun 10: 1373, 2019.
120. Courjal F, Cuny M, Simony-Lafontaine J, Louason G, Speiser P, Zeillinger R, Rodriguez C and Theillet C: Mapping of DNA amplifications at 15 chromosomal localizations in 1875 breast tumors: Definition of phenotypic groups. Cancer Res 57: 4360-4367, 1997.
121. Sharpe R, Pearson A, Herrera-Abreu MT, Johnson D, Mackay A, Welti JC, Natrajan R, Reynolds AR, Reis-Filho JS, Ashworth A and Turner NC: FGFR signaling promotes the growth of triple-negative and basal-like breast cancer cell lines both in vitro and in vivo. Clin Cancer Res 17: 5275-5286, 2011.
122. Mao P, Cohen O, Kowalski KJ, Kusiel JG, Buendia-Buendia JE, Cuoco MS, Exman P, Wander SA, Waks AG, Nayar U, *et al*: Acquired FGFR and FGF alterations confer resistance to estrogen receptor (ER) targeted therapy in ER+ metastatic breast cancer. Clin Cancer Res 26: 5974-5989, 2020.
123. Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, Natrajan R, Marchio C, Iorns E, Mackay A, *et al*: FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 70: 2085-2094, 2010.
124. Ersahin T, Tuncbag N and Cetin-Atalay R: The PI3K/AKT/mTOR interactive pathway. Mol Biosyst 11: 1946-1954, 2015.
125. Cai Z, Wang J, Li Y, Shi Q, Jin L, Li S, Zhu M, Wang Q, Wong LL, Yang W, *et al*: Overexpressed Cyclin D1 and CDK4 proteins are responsible for the resistance to CDK4/6 inhibitor in breast cancer that can be reversed by PI3K/mTOR inhibitors. Sci China Life Sci 66: 94-109, 2023.
126. Hobbs GA, Wittinghofer A and Der CJ: Selective targeting of the KRAS G12C mutant: Kicking KRAS when it's down. Cancer Cell 29: 251-253, 2016.
127. Luangdilok S, Wanchaijiraboon P, Chantranuwatana P, Teerapakpinyo C, Shuangshoti S and Sriuranpong V: Cyclin D1 expression as a potential prognostic factor in advanced KRAS-mutant non-small cell lung cancer. Transl Lung Cancer Res 8: 959-966, 2019.
128. Tran KA, Cheng MY, Mitra A, Ogawa H, Shi VY, Olney LP, Kloxin AM and Maverakis E: MEK inhibitors and their potential in the treatment of advanced melanoma: The advantages of combination therapy. Drug Des Devel Ther 10: 43-52, 2016.
129. Finn RS, Aleshin A and Slamon DJ: Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. Breast Cancer Res 18: 17, 2016.
130. Alves CL, Elias D, Lyng M, Bak M, Kirkegaard T, Lykkesfeldt AE and Ditzel HJ: High CDK6 protects cells from Fulvestrant-mediated apoptosis and is a predictor of resistance to fulvestrant in estrogen Receptor-positive metastatic breast cancer. Clin Cancer Res 22: 5514-5526, 2016.
131. Shaulian E and Karin M: AP-1 in cell proliferation and survival. Oncogene 20: 2390-2400, 2001.
132. Shen Q, Uray IP, Li Y, Zhang Y, Hill J, Xu XC, Young MR, Gunther EJ, Hilsenbeck SG, Colburn NH, *et al*: Targeting the activator protein 1 transcription factor for the prevention of estrogen receptor-negative mammary tumors. Cancer Prev Res (Phila) 1: 45-55, 2008.
133. Hydbring P, Castell A and Larsson LG: MYC modulation around the CDK2/p27/SKP2 axis. Genes (Basel) 8: 174, 2017.
134. Takahashi M, Tokunaga E, Mori J, Tanizawa Y, van der Walt JS, Kawaguchi T, Goetz MP and Toi M: Japanese subgroup analysis of the phase 3 MONARCH 3 study of abemaciclib as initial therapy for patients with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer. Breast Cancer 29: 174-184, 2022.
135. Hamilton E, Cortes J, Ozyilkan O, Chen SC, Petrakova K, Manikhas A, Jerusalem G, Hegg R, Huober J, Zhang W, *et al*: nextMONARCH Phase 2 randomized clinical trial: Overall survival analysis of abemaciclib monotherapy or in combination with tamoxifen in patients with endocrine-refractory HR+, HER2-metastatic breast cancer. Breast Cancer Res Treat 195: 55-64, 2022.
136. Goetz MP, Hamilton EP, Campone M, Hurvitz SA, Cortes J, Johnston S, Llombart-Cussac A, Kaufman PA, Toi M, Jerusalem G, *et al*: Landscape of baseline and acquired genomic alterations in circulating tumor DNA with abemaciclib alone or with endocrine therapy in advanced breast cancer. Clin Cancer Res 30: 2233-2244, 2024.
137. Pandey K, Park N, Park KS, Hur J, Cho YB, Kang M, An HJ, Kim S, Hwang S and Moon YW: Combined CDK2 and CDK4/6 inhibition overcomes palbociclib resistance in breast cancer by enhancing senescence. Cancers (Basel) 12: 3566, 2020.

138. Glaviano A, Wander SA, Baird RD, Yap KC, Lam HY, Toi M, Carbone D, Geoerger B, Serra V, Jones RH, *et al*: Mechanisms of sensitivity and resistance to CDK4/CDK6 inhibitors in hormone receptor-positive breast cancer treatment. Drug Resist Updat 76: 101103, 2024.
139. Mouron S, Manso L, Caleiras E, Rodríguez-Peralto JL, Rueda OM, Caldas C, Colomer R, Quintela-Fandino M and Bueno MJ: FGFR1 amplification or overexpression and hormonal resistance in luminal breast cancer: Rationale for a triple blockade of ER, CDK4/6, and FGFR1. Breast Cancer Res 23: 21, 2021.
140. Wang N, Ma T and Yu B: Targeting epigenetic regulators to overcome drug resistance in cancers. Signal Transduct Target Ther 8: 69, 2023.
141. Xue Y and Zhai J: Strategy of combining CDK4/6 inhibitors with other therapies and mechanisms of resistance. Int J Clin Exp Pathol 17: 189-207, 2024.
142. Manohar PM and Davidson NE: Updates in endocrine therapy for metastatic breast cancer. Cancer Biol Med 19: 202-212, 2022.
143. Finn RS, Dieras V, Rugo HS, Joy AA, Moulder SL, Walshe JM, Mukai H, Shparyk YV, Park IH, Mori A, *et al*: Palbociclib (PAL) + letrozole (L) as first-line (1L) therapy (tx) in estrogen receptor-positive (ER+)/human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (ABC): Efficacy and safety across patient (pt) subgroups. J Clin Oncol 35: 1039, 2017.
144. Kalinsky K, Accordino MK, Chiuzan C, Mundi PS, Trivedi MS, Novik Y, Tiersten A, Raptis G, Baer LN, Oh SY, *et al*: A randomized, phase II trial of fulvestrant or exemestane with or without ribociclib after progression on anti-estrogen therapy plus cyclin-dependent kinase 4/6 inhibition (CDK 4/6i) in patients (pts) with unresectable or hormone receptor-positive (HR+), HER2-negative metastatic breast cancer (MBC): MAINTAIN trial. J Clin Oncol 40: LBA1004, 2022.
145. Bidard FC, Kaklamani VG, Neven P, Streich G, Montero AJ, Forget F, Mouret-Reynier MA, Sohn JH, Taylor D, Harnden KK, *et al*: Elacestrant (oral selective estrogen receptor degrader) versus standard endocrine therapy for Estrogen Receptor-positive, human epidermal growth factor receptor 2-Negative advanced breast cancer: Results from the randomized phase III EMERALD Trial. J Clin Oncol 40: 3246-3256, 2022.
146. Oliveira M, Pominchuck D, Nowecki Z, Hamilton E, Kulyaba Y, Andabekov T, Hotko Y, Melkadze T, Nemsadze G, Neven P, *et al*: Abstract GS3-02: GS3-02 Camizestrant, a next generation oral SERD vs fulvestrant in post-menopausal women with advanced ER-positive HER2-negative breast cancer: Results of the randomized, multi-dose Phase 2 SERENA-2 trial. Cancer Res 83: GS3-02, 2023.
147. Research C for DE and: FDA approves elacestrant for ER-positive, HER2-negative, ESR1-mutated advanced or metastatic breast cancer. FDA, 2023.
148. Magge T, Rajendran S, Brufsky AM and Foldi J: CDK4/6 inhibitors: The Devil is in the detail. Curr Oncol Rep 26: 665-678, 2024.
149. Sonke GS, Van Ommen-Nijhof A, Wortelboer N, Noort VVD, Swinkels AC, Blommestein HM, Beeker A, Beelen K, Hamming LC, Heijns JB, *et al*: Primary outcome analysis of the phase 3 SONIA trial (BOOG 2017-03) on selecting the optimal position of cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors for patients with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). J Clin Oncol 41: LBA1000, 2023.
150. Sonke GS, van Ommen-Nijhof A, Wortelboer N, van der Noort V, Swinkels ACP, Blommestein HM, Guerrero Paez C, Mol L, Beeker A, Beelene K, *et al*: Early versus deferred use of CDK4/6 inhibitors in advanced breast cancer. Nature 636: 474-480, 2024.
151. Ogata R, Kishino E, Saitoh W, Koike Y and Kurebayashi J: Resistance to cyclin-dependent kinase (CDK) 4/6 inhibitors confers cross-resistance to other CDK inhibitors but not to chemotherapeutic agents in breast cancer cells. Breast Cancer 28: 206-215, 2021.
152. Rugo HS, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, Trédan O, Ciruelos E, Dalenc F, Gómez Pardo P, *et al*: Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): A randomised, open-label, multicentre, phase 3 trial. Lancet 402: 1423-1433, 2023.
153. Cao YN, Zheng LL, Wang D, Liang XX, Gao F and Zhou XL: Recent advances in microtubule-stabilizing agents. Eur J Med Chem 143: 806-828, 2018.
154. Cretella D, Fumarola C, Bonelli M, Alfieri R, La Monica S, Digiacomo G, Cavazzoni A, Galetti M, Generali D and Petronini PG: Pre-treatment with the CDK4/6 inhibitor palbociclib improves the efficacy of paclitaxel in TNBC cells. Sci Rep 9: 13014, 2019.
155. Zhang XH, Cheng Y, Shin JY, Kim JO, Oh JE and Kang JH: A CDK4/6 inhibitor enhances cytotoxicity of paclitaxel in lung adenocarcinoma cells harboring mutant KRAS as well as wild-type KRAS. Cancer Biol Ther 14: 597-605, 2013.
156. Kim JY, Jayne LA, Bai Y, Feng MJHH, Clark MA, Chung S, W Christman J, Cianciolo RE and Pabla NS: Ribociclib mitigates cisplatin-associated kidney injury through retinoblastoma-1 dependent mechanisms. Biochem Pharmaco 177: 113939, 2020.
157. Liu M, Cui L, Li X, Xia C, Li Y, Wang R, Ren F, Liu H and Chen J: PD-0332991 combined with cisplatin inhibits nonsmall cell lung cancer and reversal of cisplatin resistance. Thorac Cancer 12: 924-931, 2021.
158. Ma CX, Gao F, Luo J, Northfelt DW, Goetz M, Forero A, Hoog J, Naughton M, Ademuyiwa F and Suresh R: NeoPalAna: Neoadjuvant palbociclib, a Cyclin-dependent kinase 4/6 inhibitor, and anastrozole for clinical stage 2 or 3 estrogen receptor-positive breast cancer. Clin Cancer Res 23: 4055-4065, 2017.
159. Chow LWC, Morita S, Chow CYC, Ng WK and Toi M: Neoadjuvant palbociclib on ER+ breast cancer (N007): Clinical response and EndoPredict's value. Endocr Relat Cancer 25: 123-130, 2018.
160. Prat A, Saura C, Pascual T, Hernando C, Muñoz M, Paré L, González Farré B, Fernández PL, Galván P, Chic N, *et al*: Ribociclib plus letrozole versus chemotherapy for postmenopausal women with hormone receptor-positive, HER2-negative, luminal B breast cancer (CORALLEEN): An open-label, multicentre, randomised, phase 2 trial. Lancet Oncol 21: 33-43, 2020.
161. Delaloge S, Dureau S, D'Hondt V, Desmoulins I, Heudel PE, Duhoux FP, Levy C, Lerebours F, Mouret-Reynier MA, Dalenc F, *et al*: Survival outcomes after neoadjuvant letrozole and palbociclib versus third generation chemotherapy for patients with high-risk oestrogen receptor-positive HER2-negative breast cancer. Eur J Cancer 166: 300-308, 2022.
162. Bonelli M, La Monica S, Fumarola C and Alfieri R: Multiple effects of CDK4/6 inhibition in cancer: From cell cycle arrest to immunomodulation. Biochem Pharmacol 170: 113676, 2019.
163. Zhang S, Xu Q, Sun W, Zhou J and Zhou J: Immunomodulatory effects of CDK4/6 inhibitors. Biochim Biophys Acta Rev Cancer 1878: 188912, 2023.
164. De Angelis C, Fu X, Cataldo ML, Nardone A, Pereira R, Veeraraghavan J, Nanda S, Qin L, Sethunath V, Wang T, *et al*: Activation of the IFN signaling pathway is associated with resistance to CDK4/6 inhibitors and immune checkpoint activation in ER-Positive breast cancer. Clin Cancer Res 27: 4870-4882, 2021.
165. Wang Q, Guldner IH, Golomb SM, Sun L, Harris JA, Lu X and Zhang S: Single-cell profiling guided combinatorial immunotherapy for fast-evolving CDK4/6 inhibitor-resistant HER2-positive breast cancer. Nat Commun 10: 3817, 2019.
166. Wu CC, Wang YA, Livingston JA, Zhang J and Futreal PA: Prediction of biomarkers and therapeutic combinations for anti-PD-1 immunotherapy using the global gene network association. Nat Commun 13: 42, 2022.
167. Jang HJ, Truong CY, Lo EM, Holmes HM, Ramos D, Ramineni M, Lee JS, Wang DY, Pietropaolo M, Ripley RT, *et al*: Inhibition of cyclin dependent kinase 4/6 overcomes primary resistance to programmed cell death 1 blockade in malignant mesothelioma. Ann Thorac Surg 114: 1842-1852, 2022.
168. Litchfield K, Reading JL, Puttick C, Thakkar K, Abbosh C, Bentham R, Watkins TBK, Rosenthal R, Biswas D, Rowan A, *et al*: Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. Cell 184: 596-614.e14, 2021.
169. Jerby-Arnon L, Shah P, Cuoco MS, Rodman C, Su MJ, Melms JC, Leeson R, Kanodia A, Mei S, Lin JR, *et al*: A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. Cell 175: 984-997.e24, 2018.
170. Rugo HS, Kabos P, Beck JT, Jerusalem G, Wildiers H, Sevillano E, Paz-Ares L, Chisamore MJ, Chapman SC, Hossain AM, *et al*: Abemaciclib in combination with pembrolizumab for HR+, HER2-metastatic breast cancer: Phase 1b study. NPJ Breast Cancer 8: 118, 2022.

171. Yuan Y, Lee JS, Yost SE, Frankel PH, Ruel C, Egelston CA, Guo W, Padam S, Tang A, Martinez N, *et al*: Phase I/II trial of palbociclib, pembrolizumab and letrozole in patients with hormone receptor-positive metastatic breast cancer. Eur J Cancer 154: 11-20, 2021.
172. Lee EK, Esselen KM, Kolin DL, Lee LJ, Matulonis UA and Konstantinopoulos PA: Combined CDK4/6 and PD-1 inhibition in refractory SMARCA4-deficient Small-cell carcinoma of the ovary, hypercalcemic type. JCO Precis Oncol 4: 736-742, 2020.
173. Xue Y, Meehan B, Fu Z, Wang XQD, Fiset PO, Rieker R, Levins C, Kong T, Zhu X, Morin G, *et al*: SMARCA4 loss is synthetic lethal with CDK4/6 inhibition in non-small cell lung cancer. Nat Commun 10: 557, 2019.
174. Bose S, Lee T, Choi S, Fazlollahi L, Rasiej MJ, Schwartz GK and Ingham M: CDK4/6 inhibition with Anti-PD-1 checkpoint blockade induces major response in aggressive classic kaposi sarcoma after previous progression on Anti-PD-1 alone. JCO Precis Oncol 6: e2100550, 2022.
175. Daniel D, Kuchava V, Bondarenko I, Ivashchuk O, Reddy S, Jaal J, Kudaba I, Hart L, Matitashvili A, Pritchett Y, *et al*: Trilaciclib prior to chemotherapy and atezolizumab in patients with newly diagnosed extensive-stage small cell lung cancer: A multicentre, randomised, double-blind, placebo-controlled Phase II trial. Int J Cancer 148: 2557-2570, 2021.
176. Mayer EL, Ren Y, Wagle N, Mahtani R, Ma C, DeMichele A, Cristofanilli M, Meisel J, Miller KD, Jolly T, *et al*: Abstract GS3-06: GS3-06 Palbociclib After CDK4/6i and endocrine therapy (PACE): A randomized phase II study of fulvestrant, palbociclib, and avelumab for endocrine Pre-treated ER+/HER2-metastatic breast cancer. Cancer Res 83: GS3-06, 2023.
177. Jerusalem G, Prat A, Salgado R, Reinisch M, Saura C, Ruiz-Borrego M, Nikolinakos P, Ades F, Filian J, Huang N, *et al*: Neoadjuvant nivolumab + palbociclib + anastrozole for oestrogen receptor-positive/human epidermal growth factor receptor 2-negative primary breast cancer: Results from CheckMate 7A8. Breast 72: 103580, 2023.
178. Dennis MJ, Sacco AG, Qi Y, Bykowski J, Pittman E, Chen R, Messer K, Cohen EEW and Gold KA: A phase I study of avelumab, palbociclib, and cetuximab in patients with recurrent or metastatic head and neck squamous cell carcinoma. Oral Oncol 135: 106219, 2022.
179. Pujol JL, Vansteenkiste J, Paz-Ares Rodríguez L, Gregorc V, Mazieres J, Awad M, Jänne PA, Chisamore M, Hossain AM, Chen Y and Beck JT: Abemaciclib in combination with pembrolizumab for stage IV KRAS-mutant or squamous NSCLC: A phase 1b study. JTO Clin Res Rep 2: 100234, 2021.
180. Patnaik A, Yap TA, Chung HC, de Miguel MJ, Bang YJ, Lin CC, Su WC, Italiano A, Chow KH, Szpurka AM, *et al*: Safety and clinical activity of a new Anti-PD-L1 antibody as monotherapy or combined with targeted therapy in advanced solid tumors: The PACT Phase Ia/Ib trial. Clin Cancer Res 27: 1267-1277, 2021.
181. Garrido-Castro AC and Goel S: CDK4/6 inhibition in breast cancer: Mechanisms of response and treatment failure. Curr Breast Cancer Rep 9: 26-33, 2017.
182. Clark AS, Makhlin I and DeMichele A: Setting the pick: Can PI3K inhibitors circumvent CDK4/6 inhibitor resistance? Clin Cancer Res 27: 371-373, 2021.
183. Masuda J, Sakai H, Tsurutani J, Tanabe Y, Masuda N, Iwasa T, Takahashi M, Futamura M, Matsumoto K, Aogi K, *et al*: Efficacy, safety, and biomarker analysis of nivolumab in combination with abemaciclib plus endocrine therapy in patients with HR-positive HER2-negative metastatic breast cancer: A phase II study (WJOG11418B NEWFLAME trial). J Immunother Cancer 11: e007126, 2023.
184. Bedard PL, Hyman DM, Davids MS and Siu LL: Small molecules, big impact: 20 years of targeted therapy in oncology. Lancet 395: 1078-1088, 2020.
185. Gómez Tejeda Zañudo J, Barroso-Sousa R, Jain E, Jin Q, Li T, Buendia-Buendia JE, Pereslete A, Abravanel DL, Ferreira AR, Wrabel E, *et al*: Exemestane plus everolimus and palbociclib in metastatic breast cancer: Clinical response and genomic/transcriptomic determinants of resistance in a phase I/II trial. Nat Commun 15: 2446, 2024.
186. Jhaveri KL, Accordino MK, Bedard PL, Cervantes A, Gambardella V, Hamilton E, Italiano A, Kalinsky K, Krop IE, Oliveira M, *et al*: Phase I/Ib trial of inavolisib plus palbociclib and endocrine therapy for PIK3CA-Mutated, hormone Receptor-positive, human epidermal growth factor Receptor 2-Negative advanced or metastatic breast cancer. J Clin Oncol 42: 3947-3956, 2024.
187. Yardley DA, Noguchi S, Pritchard KI, Burris HA III, Baselga J, Gnant M, Hortobagyi GN, Campone M, Pistilli B, Piccart M, *et al*: Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2 final progression-free survival analysis. Adv Ther 30: 870-884, 2013.
188. Pascual J, Lim JSJ, Macpherson IR, Armstrong AC, Ring A, Okines AFC, Cutts RJ, Herrera-Abreu MT, Garcia-Murillas I, Pearson A, *et al*: Triplet therapy with palbociclib, taselisib, and fulvestrant in PIK3CA-mutant breast cancer and doublet palbociclib and taselisib in Pathway-mutant solid cancers. Cancer Discov 11: 92-107, 2021.
189. Wander SA, Juric D, Supko JG, Micalizzi DS, Spring S, Vidula N, Beeler M, Habin KR, Viscosi E, Fitzgerald DM, *et al*: Phase Ib trial to evaluate safety and anti-tumor activity of the AKT inhibitor, ipatasertib, in combination with endocrine therapy and a CDK4/6 inhibitor for patients with hormone receptor positive (HR+)/HER2 negative metastatic breast cancer (MBC) (TAKTIC). J Clin Oncol 38: 1066, 2020.
190. Huang J, Zheng L, Sun Z and Li J: CDK4/6 inhibitor resistance mechanisms and treatment strategies (review). Int J Mol Med 50: 128, 2022.
191. Piccart M, Hortobagyi GN, Campone M, Pritchard KI, Lebrun F, Ito Y, Noguchi S, Perez A, Rugo HS, Deleu I, *et al*: Everolimus plus exemestane for hormone-receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: Overall survival results from BOLERO-2†. Ann Oncol 25: 2357-2362, 2014.
192. Lord CJ and Ashworth A: PARP inhibitors: Synthetic lethality in the clinic. Science 355: 1152-1158, 2017.
193. Guney Eskiler G, Ozman Z, Haciefendi A and Cansaran-Duman D: Novel combination treatment of CDK 4/6 inhibitors with PARP inhibitors in triple negative breast cancer cells. Naunyn Schmiedebergs Arch Pharmacol 396: 1031-1041, 2023.
194. Wu C, Peng S, Pilié PG, Geng C, Park S, Manyam GC, Lu Y, Yang G, Tang Z, Kondraganti S, *et al*: PARP and CDK4/6 inhibitor combination therapy induces apoptosis and suppresses neuroendocrine differentiation in prostate cancer. Mol Cancer Ther 20: 1680-1691, 2021.
195. Zhu X, Chen L, Huang B, Li X, Yang L, Hu X, Jiang Y, Shao Z and Wang Z: Efficacy and mechanism of the combination of PARP and CDK4/6 inhibitors in the treatment of triple-negative breast cancer. J Exp Clin Cancer Res 40: 122, 2021.
196. Curtin NJ and Szabo C: Poly(ADP-ribose) polymerase inhibition: Past, present and future. Nat Rev Drug Discov 19: 711-736, 2020.
197. Jiang Z, Li W, Hu X, Zhang Q, Sun T, Cui S, Wang S, Ouyang Q, Yin Y, Geng C, *et al*: Tucidinostat plus exemestane for postmenopausal patients with advanced, hormone receptor-positive breast cancer (ACE): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20: 806-815, 2019.
198. Reeves C: San Antonio breast cancer symposium 2021. Lancet Oncol 23: e18, 2022.
199. Lok SW, Whittle JR, Vaillant F, The CE, Lo LL, Policheni AN, Bergin ART, Desai J, Ftouni S, Gandolfo LC, *et al*: A phase Ib dose-escalation and expansion study of the BCL2 inhibitor venetoclax combined with tamoxifen in ER and BCL2-positive metastatic breast cancer. Cancer Discov 9: 354-369, 2019.
200. Lindeman GJ, Fernando TM, Bowen R, Jerzak KJ, Song X, Decker T, Boyle F, McCune S, Armstrong A, Shannon C, *et al*: VERONICA: Randomized phase II study of fulvestrant and venetoclax in ER-positive metastatic breast cancer Post-CDK4/6 inhibitors-efficacy, safety, and biomarker results. Clin Cancer Res 28: 3256-3267, 2022.
201. Benvenuti C, Grinda T, Rassy E, Dixon-Douglas J, Ribeiro JM, Zambelli A, Santoro A and Pistilli B: Unveiling the potential of Cyclin-dependent kinases 4 and 6 inhibitors beyond progression in hormone receptor positive/human epidermal growth factor negative advanced breast cancer-a clinical review. Curr Treat Options Oncol 25: 1517-1537, 2024.
202. Kubeczko M, Jarząb M, Gabryś D, Krzywon A, Cortez AJ and Xu AJ: Safety and feasibility of CDK4/6 inhibitors treatment combined with radiotherapy in patients with HR-positive/HER2-negative breast cancer. A systematic review and meta-analysis. Radiother Oncol 187: 109839, 2023.
203. Lee CL, Oh P, Xu ES, Ma Y, Kim Y, Daniel AR and Kirsch DG: Blocking Cyclin-dependent kinase 4/6 during single dose versus fractionated radiation therapy leads to opposite effects on acute gastrointestinal toxicity in mice. Int J Radiat Oncol Biol Phys 102: 1569-1576, 2018.

204. Naz S, Sowers A, Choudhuri R, Wissler M, Gamson J, Mathias A, Cook JA and Mitchell JB: Abemaciclib, a selective CDK4/6 inhibitor, enhances the radiosensitivity of Non-small cell lung cancer in vitro and in vivo. Clin Cancer Res 24: 3994-4005, 2018.
205. Hagen KR, Zeng X, Lee MY, Tucker Kahn S, Harrison Pitner MK, Zaky SS, Liu Y, O'Regan RM, Deng X and Saavedra HI: Silencing CDK4 radiosensitizes breast cancer cells by promoting apoptosis. Cell Div 8: 10, 2013.
206. Whittaker S, Madani D, Joshi S, Chung SA, Johns T, Day B, Khasraw M and McDonald KL: Combination of palbociclib and radiotherapy for glioblastoma. Cell Death Discov 3: 17033, 2017.
207. Huang CY, Hsieh FS, Wang CY, Chen LJ, Chang SS, Tsai MH, Hung MH, Kuo CW, Shih CT, Chao TI and Chen KF: Palbociclib enhances radiosensitivity of hepatocellular carcinoma and cholangiocarcinoma via inhibiting ataxia telangiectasia-mutated kinase-mediated DNA damage response. Eur J Cancer 102: 10-22, 2018.
208. Slamon DJ, Stroyakovskiy D, Yardley DA, Huang C, Fasching PA, Crown J, Bardia A, Chia S, Im S, Martin M, *et al*: Ribociclib and endocrine therapy as adjuvant treatment in patients with HR+/HER2-early breast cancer: Primary results from the phase III NATALEE trial. J Clin Oncol 41: LBA500, 2023.
209. Becherini C, Visani L, Caini S, Bhattacharya IS, Kirby AM, Nader Marta G, Morgan G, Salvestrini V, Coles CE, Cortes J, *et al*: Safety profile of cyclin-dependent kinase (CDK) 4/6 inhibitors with concurrent radiation therapy: A systematic review and meta-analysis. Cancer Treat Rev 119: 102586, 2023.
210. Franco R, Cao JQ, Yassa M and Hijal T: Safety of CDK4/6 inhibitors combined with radiotherapy in patients with metastatic breast cancer: A Review of the literature. Curr Oncol 30: 5485-5496, 2023.
211. Ratosa I, Orazem M, Scoccimarro E, Steinacher M, Dominici L, Aquilano M, Cerbai C, Desideri I, Ribnikar D, Marinko T, *et al*: Cyclin-Dependent Kinase 4/6 inhibitors combined with radiotherapy for patients with metastatic breast cancer. Clin Breast Cancer 20: 495-502, 2020.
212. Kawamoto T, Shikama N, Imano N, Kubota H, Kosugi T, Sekii S, Harada H, Yamada K, Naoi Y, Miyazawa K, *et al*: Incidence of and risk factors for non-hematologic toxicity with combined radiotherapy and CDK4/6 inhibitors in metastatic breast cancer using dose-volume parameters analysis: A multicenter cohort study. Breast Cancer 30: 282-292, 2023.
213. Hashizume R, Zhang A, Mueller S, Prados MD, Lulla RR, Goldman S, Saratsis AM, Mazar AP, Stegh AH, Cheng SY, *et al*: Inhibition of DNA damage repair by the CDK4/6 inhibitor palbociclib delays irradiated intracranial atypical teratoid rhabdoid tumor and glioblastoma xenograft regrowth. Neuro Oncol 18: 1519-1528, 2016.
214. Pesch AM, Hirsh NH, Chandler BC, Michmerhuizen AR, Ritter CL, Androsiglio MP, Wilder-Romans K, Liu M, Gersch CL, Larios JM, *et al*: Short-term CDK4/6 inhibition radiosensitizes estrogen receptor-positive breast cancers. Clin Cancer Res 26: 6568-6580, 2020.
215. Meattini I, Livi L, Lorito N, Becherini C, Bacci M, Visani L, Fozza A, Belgioia L, Loi M, Mangoni M, *et al*: Integrating radiation therapy with targeted treatments for breast cancer: From bench to bedside. Cancer Treat Rev 108: 102417, 2022.
216. Zhang X, Zhu L, Zhang H, Chen S and Xiao Y: CAR-T cell therapy in hematological malignancies: Current opportunities and challenges. Front Immunol 13: 927153, 2022.
217. Zhang ZZ, Wang T, Wang XF, Zhang YQ, Song SX and Ma CQ: Improving the ability of CAR-T cells to hit solid tumors: Challenges and strategies. Pharmacol Res 175: 106036, 2022.
218. Lelliott EJ, Kong IY, Zethoven M, Ramsbottom KM, Martelotto LG, Meyran D, Zhu JJ, Costacurta M, Kirby L, Sandow JJ, *et al*: CDK4/6 inhibition promotes antitumor immunity through the induction of T-cell memory. Cancer Discov 11: 2582-2601, 2021.
219. Tripathy D, Im SA, Colleoni M, Franke F, Bardia A, Harbeck N, Hurvitz SA, Chow L, Sohn J, Lee KS, *et al*: Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): A randomised phase 3 trial. Lancet Oncol 19: 904-915, 2018.
220. Turner NC, Ro J, André F, Loi S, Verma S, Iwata H, Harbeck N, Loibl S, Huang Bartlett C, Zhang K, *et al*: Palbociclib in Hormone-Receptor-positive advanced breast cancer. N Engl J Med 373: 209-219, 2015.
221. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, Campone M, Petrakova K, Blackwell KL, Winer EP, *et al*: Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Ann Oncol 29: 1541-1547, 2018.
222. Rugo HS, Huober J, García-Sáenz JA, Masuda N, Sohn JH, Andre VAM, Barriga S, Cox J and Goetz M: Management of Abemaciclib-associated adverse events in patients with hormone Receptor-positive, human epidermal growth factor receptor 2-Negative advanced breast cancer: Safety analysis of MONARCH 2 and MONARCH 3. Oncologist 26: e53-e65, 2021.
223. Spring LM, Zangardi ML, Moy B and Bardia A: Clinical management of potential toxicities and drug interactions related to Cyclin-Dependent kinase 4/6 inhibitors in breast cancer: Practical considerations and recommendations. Oncologist 22: 1039-1048, 2017.
224. Asghar U, Witkiewicz AK, Turner NC and Knudsen ES: The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 14: 130-146, 2015.
225. Hassanzadeh A, Shomali N, Kamrani A, Soltani-Zangbar MS, Nasiri H and Akbari M: Cancer therapy by cyclin-dependent kinase inhibitors (CDKIs): Bench to bedside. EXCLI J 23: 862-882, 2024.
226. Zheng M, Zhang XY, Chen W, Xia F, Yang H, Yuan K and Yang P: Molecules inducing specific cyclin-dependent kinase degradation and their possible use in cancer therapy. Future Med Chem 16: 369-388, 2024.
227. Majeski H, Okano A, Pasis A, Carlson C, Shakya A, Huang S, Hoskote Chourasia A, Fung LM and Liu Q: Discovery of CDK4/6 bifunctional degraders for ER+/HER2-breast cancer. J Clin Oncol 14: 1083, 2023.
228. Schott AF, Hurvitz S, Ma C, Hamilton E, Nanda R, Zahrah G, Hunter N, Tan AR, Telli M, Mesias JA, *et al*: Abstract GS3-03: GS3-03 ARV-471, a PROTAC® estrogen receptor (ER) degrader in advanced ER-positive/human epidermal growth factor receptor 2 (HER2)-negative breast cancer: Phase 2 expansion (VERITAC) of a phase 1/2 study. Cancer Res 83 (Suppl 5): GS3-03, 2023.
229. Bruls R: Treatment options beyond CDK4/6 inhibition. Medical Conferences, 2023.
230. Fassl A, Brain C, Abu-Remaileh M, Stukan I, Butter D, Stepien P, Feit AS, Bergholz J, Michowski W, Otto T, *et al*: Increased lysosomal biomass is responsible for the resistance of triple-negative breast cancers to CDK4/6 inhibition. Sci Adv 6: eabb2210, 2020.
231. Hassan MA-K and Ates-Alagoz Z: Cyclin-dependent kinase 4/6 inhibitors against breast cancer. Mini Rev Med Chem 23: 412-428, 2023.
232. Lv S, Yang J, Lin J, Huang X, Zhao H, Zhao C and Yang L: CDK4/6 inhibitors in lung cancer: Current practice and future directions. Eur Respir Rev 33: 230145, 2024.
233. Du Q, Guo X, Wang M, Li Y, Sun X and Li Q: The application and prospect of CDK4/6 inhibitors in malignant solid tumors. J Hematol Oncol 13: 41, 2020.
234. Sun M, Dong L, Wang Y, Liu C, Du J, Wang B, Xing B, Yao X, Ren Y and Zhou X: The role of targeting CDK4/6 in cancer immunotherapy. Holist Integ Oncol 3: 32, 2024.