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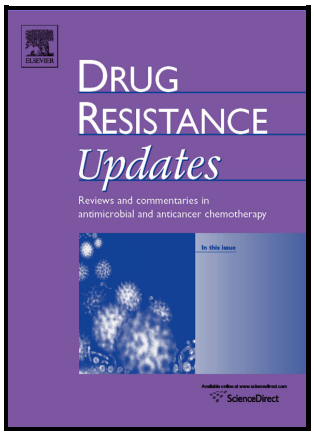

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MECHANISMS OF SENSITIVITY AND RESISTANCE TO CDK4/CDK6 INHIBITORS IN HORMONE RECEPTOR-POSITIVE BREAST CANCER TREATMENT

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Abstract

Cell cycle dysregulation is a hallmark of cancer that promotes eccessive cell division. Cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) are key molecules in the G1-to-S phase cell cycle transition and are crucial for the onset, survival, and progression of breast cancer (BC). Small-molecule CDK4/CDK6 inhibitors (CDK4/6i) block phosphorylation of tumor suppressor Rb and thus restrain susceptible BC cells in G1 phase. Three CDK4/6i are approved for the first-line treatment of patients with advanced/metastatic hormone receptor-positive (HR^+)/human epidermal growth factor receptor 2-negative ($HER2^-$) BC in

combination with endocrine therapy (ET). Though this has improved the clinical outcomes for survival of BC patients, there is no established standard next-line treatment to tackle drug resistance. Recent studies suggest that CDK4/6i can modulate other distinct effects in both BC and breast stromal compartments, which may provide new insights into aspects of their clinical activity. This review describes the biochemistry of the CDK4/6-Rb-E2F pathway in HR^+ BC, then discusses how CDK4/6i can trigger other effects in BC/breast stromal compartments, and finally outlines the mechanisms of CDK4/6i resistance that have emerged in recent preclinical studies and clinical cohorts, emphasizing the impact of these findings on novel therapeutic opportunities in BC.

Keywords

Cyclin-dependent kinases 4 (CDK4); cyclin-dependent kinases 6 (CDK6); CDK4/CDK6 inhibitors (CDK4/6i); CDK4/6i resistance; hormone receptor-positive (HR^+) breast cancer; endocrine therapy (ET).

1. Introduction

The mammalian cell cycle is precisely controlled to ensure correct genome duplication and its dysregulation often contributes to oncogenesis (1). In recent years, specific small-molecule cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) inhibitors (CDK4/6i) have revolutionized the standard of care for advanced hormone receptor-positive (HR^+)/HER2-negative ($HER2^-$) breast cancer (BC) patients (2) (3) (4). Even though endocrine therapy (ET) has been the main systemic treatment for estrogen-receptor positive (ER^+) and $HER2^-$ BC (5), patients still often display innate and acquired drug resistance (6) (7). Importantly, addition of CDK4/6i to ET effectively suppresses cell proliferation, reduces tumor progression, and improves patient outcomes (8). Thus, co-treatment of CDK4/6i and ET is considered the current standard of care for first-line therapy in recurrent unresectable or metastatic advanced $ER^+/HER2^-$ BC patients (9). Accordingly, the small-molecule CDK4/6i palbociclib, ribociclib, and abemaciclib have been Food and Drug Administration (FDA)-approved in combination with an aromatase inhibitor (AI) for $HR^+/HER2^-$ BC (10). In addition, combined treatment with fulvestrant, a selective estrogen receptor degrader (SERD), has exhibited positive results for $HR^+/HER2^-$ BC patients after progression on adjuvant AI (11). Abemaciclib has also been uniquely FDA-approved as monotherapy in refractory advanced $HR^+/HER2^-$ BC (12) (13). Palbociclib, ribociclib, and abemaciclib (14) (15) potently suppress CDK4/6 enzymatic activity at nanomolar levels, but display higher selectivity for CDK4 compared to CDK6 (16) (17) (18) (15) (**TABLE 1**). Abemaciclib exerts broader CDK suppression and also inhibits cyclin-dependent kinase 1 (CDK1), cyclin-dependent kinase 2 (CDK2) and cyclin-dependent kinase 9 (CDK9) *in vivo* (19). Apart from blocking cancer cells in the G1 phase of the cell cycle, which is common to all three CDK4/6i, abemaciclib can also induce G2 phase arrest, in particular at concentrations of 0.3 μM and above, perhaps determined by the suppression of CDK1 and CDK2, whose activities are necessary for cell cycle progression through S-phase and mitosis (15). Additionally, abemaciclib long-term treatment also causes increased apoptosis in Rb-proficient BC cells compared to palbociclib or ribociclib (20). Generally, the safety profile of these three drugs is favorable (21) (22). Though there is a high incidence of myelosuppression, particularly neutropenia, there is a low rate of febrile neutropenia (23). Abemaciclib provokes more gastrointestinal-related toxicity compared to palbociclib and ribociclib (24) (23) possibly due to CDK9 inhibition (23). Conversely,

abemaciclib is less myelosuppressive. Notably, palbociclib and ribociclib regimens require a 1-week break at the end of each 4-week treatment cycle to permit recovery of bone marrow; whereas abemaciclib can be administered continuously (18) (15) (9). Other newer CDK4/6i are under development, including lerociclib, which has shown signals of efficacy and favorable safety profile in a phase 1/phase 2 dose escalation/expansion clinical trial (NCT02983071) in combination with fulvestrant (25). Furthermore, dalpiciclib has shown a positive impact on progression free survival (PFS) as a second-line or third-line setting via the DAWNA-1 phase 3 clinical trial (NCT03927456) (26), and in combination with either AI letrozole or anastrozole, as a first-line setting, in the DAWNA-2 phase 3 clinical trial (NCT03966898) (27). Patients with metastatic BC eventually progress on CDK4/6i to intrinsic/acquired resistance (28), for which there is no standard next-line treatment. Thus, improving our knowledge of the mechanism of resistance to CDK4/6i is crucial to establish better treatment strategies. Recent preclinical studies have shown that CDK4/6i can also induce a variety of phenotypes beyond cell cycle arrest, emphasizing that deeper insights into the mechanisms of action of these agents is required (10). In this review, we first explain the biology and role of the CDK4/6-Rb-E2F pathway in HR^+ BC, then outline how small-molecule CDK4/6i can trigger other effects in cancer/stromal compartments, and finally describe the notorious mechanisms of CDK4/6i resistance that have emerged in recent preclinical studies and clinical cohorts, highlighting possible therapeutic strategies in the context of CDK4/6i resistance. Our detailed review on CDK4/6i resistance in BC will provide comprehensive insights to clinicians and scientists regarding how to fully exploit the promise of these CDK4/6-inhibiting therapeutic drugs.

2. CDK4/6-mediated regulation of S phase entry in HR^+ breast cancer

2.1. Cyclin D-CDK4/6 signaling pathway in the cell cycle

The cell cycle (**FIGURE 1**) is characterized by accurate regulation of a myriad of cellular events. A gross departure from this precision can lead to mis-regulation and genomic instability, which often contributes to oncogenesis (29). Cell cycle control and transcription are two essential processes strictly regulated by cyclin-dependent kinases (CDKs). The human cyclin/CDK network includes up to 30 different cyclin proteins and over 20 CDKs (30). Cyclins increase during different phases of the cell cycle, guided by transcription and suppression of protein degradation, to permit cell cycle entry and progression. In turn, cell cycle-mediated transcription depends upon CDK activity (31). Though Cyclin D1/D2/D3 binding promotes partial activation of CDK4/6, complete induction requires phosphorylation by CDK-activating kinase (CAK), a trimeric protein complex comprised of cyclin H, cyclin-dependent kinase 7 (CDK7), and Mat1 subunit (32). The complexes cyclin D/CDK4 and cyclin D/CDK6 specifically promote a proliferative G1 state, priming cells for a G1-to-S phase transition (33) (34), induced by E2F transcription factors whose activity determines DNA replication-related gene expression (35). Tumor suppressor Rb forms complexes with E2Fs and downregulates numerous genes coding for key cell cycle regulators. Besides, Rb-recruited epigenetic modifiers place repressive chromatin marks at the promoters of E2F target genes (36). Commitment to replication initiation is tightly associated with induction of E2F-dependent transcription, which is activated in G1 phase and successively suppressed during S phase (37) (38). In G0 phase, the quiescent cellular state outside of the cell cycle, Rb is unphosphorylated (39). Mitogenic growth factors activate and increase the levels of cyclin D, which thereupon binds to CDK4/6. The holoenzyme complex cyclin D-CDK4/6 then interacts with p21 or p27, resulting in phosphorylation of Rb (40) (41). Recent studies suggest that cyclin D-CDK4 and cyclin D-CDK6 are not directly related to the initial inactivation of Rb, but instead, prime cells for a new cell cycle entry (from mitosis into G1 phase) by either hindering its exit from G1

phase, or promoting its entry into G1 phase (40), probably by regulating the metabolic state of cells (42) (43). In both cases, the complex cyclin D-CDK4/6-p21/p27 endorses mono-phosphorylation of Rb, which does not inactivate Rb, and consequently is inadequate to stimulate E2F-dependent transcription (44) (45). Instead, this CDK4/6-mediated mono-phosphorylation of Rb generates a temporary state between a new cell cycle entry (from mitosis into G1 phase) and cell cycle exit (33). Concurrently, in this state, accumulation of cyclin E results in the formation of the complex cyclin E/CDK2, which then inactivates Rb via hyperphosphorylation leading to activation of E2F-dependent transcription (46) (47). Since both cyclin E and cyclin A are E2F targets, this causes their hyper-accumulation, initiating a positive feedback loop with cyclin E/CDK2 further activating E2F-dependent transcription via Rb inactivation and accumulation of cyclin A/CDK2 activity, thereby driving S phase entry (48). However, considering potential redundancy between CDK2 and CDK4/6 is important in regard to irregular responses of Rb-proficient tumors to CDK4/6 inhibition. In this respect, CDK2 or CDK4/6 singly are also capable of driving proliferation. For instance, distinct Rb-proficient tumor cells can proliferate without the presence of CDK2 (49), and cells absent of CDK4/6 can continue proliferating by forming peculiar cyclin D-CDK2 complexes, thereby maintaining their ability to phosphorylate Rb (50) (51). Once S phase entry occurs, cell cycle proceeds toward S phase, G2 phase, and M phase (1). The onset of DNA replication, and thus commitment to begin S phase mainly depends on cyclin A/CDK2 activity. As cells traverse through S phase, cyclin E/E2F association gradually decreases, and conversely, cyclin A/E2F association increases (52). This triggers progression through the replication initiation commitment point (RICP), DNA replication, and a new cell cycle (53). Once DNA replication is attained, there is a post-replicative state, during which, CDK1 activity undergoes meticulous regulation to allow prompt and speedy mitotic entry. CDK1 then associates with cyclin A and cyclin B, which progressively increase in G2 phase (54). This translates into a prompt increase in CDK1 activity which drives mitotic entry (55). Upon attaining a proper level of CDK1 activity, and reaching the mitosis initiation commitment point (MICP), entry into mitosis is generated by phosphorylation of indefinite CDK1 substrates (56) (57). Finally, mitosis-associated events are mechanistically controlled predominantly by the cyclin B/CDK1 complex. Activation of the anaphase-promoting complexes then directs degradation of cyclins, which abrogates CDK activity when the two daughter cells return to interphase (58) (**FIGURE 1**).

2.2. Altered cyclin D-CDK4/6 signaling pathway in the cell cycle

2.2.1. Hyperactivity of cyclin D-CDK4/6 caused by upstream signaling pathways

Ubiquitous mechanisms without genetic alterations in the cyclin D-CDK4/6 signaling pathway can also drive CDK4/6 hyperactivity in BC, leading to upregulation of cyclin D. This is caused by high-levels of mitosis-inducing signals, disrupting the rise and fall counteract of cyclin D levels normally observed in G1 phase (59) (33) (60). Mutations deregulating the PI3K/AKT pathway augment cyclin D expression through multiple mechanisms, such as enhanced mRNA translation (61), decreased nuclear export/protein degradation (62), and derepression of cyclin D gene transcription (63); whereas the MAPK signalling pathway-mediated mutations can directly drive *CCND1* transcription (64). In line with this, downstream of both the PI3K/AKT and MAPK axes, mTOR complex 1 (mTORC1) can principally augment mRNA translation of cyclin D (65). Moreover, mutations in other signalling pathways can also enhance the activity of CDK4/6 in tumor cells, through a variety of biochemical mechanisms. For instance, ER augments *CCND1* transcription in estrogen-sensitive tumors such as BC, and β-catenin can induce transcription from the *CCND1* promoter in BC cells (51). Besides, mutations in estrogen receptor 1 (*ESR1*) are common in ER^+ BC patients who progress on ET.

Most of these mutations involve three residues (Leu-536, Tyr-537, and Asp-538) at the C-terminal helix 12 of the hormone binding domain (66), resulting in constitutive, ligand-independent ER signaling (**FIGURE 2**).

2.2.2. Intrinsic cell cycle mutation-induced hyperactivity of cyclin D-CDK4/6

Abnormal functioning of cyclin D/CDK4 and cyclin D/CDK6 are common in various human cancers, including BC (**FIGURE 2**), priming cells for S phase entry, and thus contributing to activation of E2F family of transcription factors (67), to guide cyclin E/CDK2- and downstream cyclin A/CDK2-induced cell cycle advancement from G1 phase to S phase (34). Additionally, mutations in pocket protein family (Rb, p107, and p130) (68) augment E2F-dependent transcriptional regulation, thereby endorsing cyclin E/CDK2, as well as downstream cyclin A/CDK2 activity in cancer cells. Thus, mutation-mediated CDK overactivity determines increased phosphorylation and inactivation of Rb, allowing expression of E2F-dependent genes, such as cyclin E1-encoding *CCNE1* and cyclin E2-encoding *CCNE2*. Subsequently, cyclin E binds and activates CDK2, resulting in further hyperphosphorylation of Rb and phosphorylation of several other molecules, leading to a permanent commitment to S phase entry (48). Molecular studies strongly suggest that cyclin D-CDK4/6 signaling pathway is frequently hyperactivated in HR^+ BC (69). Indeed, cyclin D1-encoding *CCND1* oncogene amplification (70), and either CDK4 amplification or loss of $p16^{INK4a}$- and $p14^{ARF}$-encoding tumor suppressor *CDKN2A* (71), have been reported in BC. Transcriptional factor estrogen receptor, the main driver of tumor growth and survival in HR^+ BC, also directly targets *CCND1* (72) (**FIGURE 2**).

3. Mechanisms of action of CDK4/6i in HR^+ breast cancer

The exact molecular mechanisms whereby FDA-approved small-molecule CDK4/6i inhibit cellular proliferation is still uncertain. These compounds interact with the ATP-binding pocket of CDK4/6, leading to competitive suppression of active CDK4/6 kinases. Nevertheless, though active CDK4/6 is commonly present in a trimer with cyclin D and p21 or cyclin D and p27 (73), *in vitro* efficacy of these compounds has been demonstrated using cyclin D-CDK4/6 dimers (16) (17) (74). Interestingly, palbociclib, ribociclib, and abemaciclib, exhibit no inhibition of active trimeric complexes *in vitro*, and consequently, it has been hypothesized that instead they may directly suppress active CDK4/6 in cells (51). Alternatively, CDK4/6i may act by binding and sequestering monomeric inoperative CDK4/6, precluding formation of cyclin D-CDK4/6-p21/p27 holoenzyme trimers, thereby freeing p21 to bind and suppress CDK2 (75). However, the hypothesis that CDK4/6i mainly act through indirect CDK2 suppression has not been tested (76), and ought to be demonstrated by showing that CDK4/6i-sensitive tumor cells are more susceptible to genetic disruption of the CDK2 kinase function than CDK4/6 kinase function (51). In addition, CDK6 exists in both thermostable and thermounstable forms; and the former exhibits resistance to current pharmacological CDK4/6i (77).

3.1. Inhibiting G1 phase/S phase CDKs

Palbociclib, ribociclib, and abemaciclib target the ATP-binding domains of CDK4 and CDK6 (78). These drugs are cytostatic and their activity leads to a G1-phase arrest in Rb-proficient luminal HR^+ BC cells (19) (**FIGURE 3A**). Interestingly, unlike palbociclib and ribociclib, treatment with abemaciclib at higher concentrations blocks cancer cells in both G1 and G2

phases (15) and activates apoptosis (20) in Rb-proficient BC cells. This characteristic may be partially due to differences in secondary targets of abemaciclib such as proto-oncogene PIM kinases (79). In addition, abemaciclib-specific targets can also include CDK1, CDK2, and CDK9, although current data does not provide significance that these kinases are fully hypophosphorylated after treatment of HR^+ BC cells (19) (80). Importantly, all these three aforementioned CDK4/6i display their strongest potency in the presence of functional Rb (81). There is still uncertainty regarding the mechanisms by which CDK4/6i exert their cytostatic activity toward BC cells. Guiley *et al* have proposed a model whereby CDK4/6-mediated arrest of cell cycle is mainly determined by indirect inhibition of CDK2. In this case, CDK4/6i primarily bind to inactive monomeric CDK4 or CDK6 instead of binding and suppressing the active cyclin D-CDK4/6-p21/27 complexes. As a result, CDK4/6i exert no blockage of endogenous CDK4 activity, but rather preclude the establishment of steady cyclin D-CDK4/6-p21/27 trimers. Consequently, this allows p21-mediated inhibition of CDK2 activity, resulting in G1-phase cell cycle arrest (75) (**FIGURE 3B**). However, Pennycook and Barr (2021) have shown that palbociclib-mediated cell cycle arrest can occur in the absence of the CDK inhibitors p21 and p27 (76). The Pack *et al* model has recently suggested that treatment with CDK4/6i promptly dissociates p21 selectively from CDK4 complexes, but unexpectedly, not from CDK6 complexes; and that the indirect inhibition of CDK2 activity is achieved by p21 but not by p27 redistribution (82). Overall, this study indicates that CDK4/6i could mediate two concurrent major roles to repress cell cycle advancement: *1)* a direct catalytic suppression of CDK4/6-induced Rb phosphorylation, independent of p21; and *2)* an indirect non-catalytic inhibition of CDK2 by displacing p21 from cycin D/CDK4/p21 trimeric complex, which is unique to CDK4-p21, and not CDK6-p27 (82) (**FIGURE 3C**). Interestingly, recent studies have identified a role for the INK4 tumor suppressor proteins in mediating resistance to CDK4/6i. Chandarlapaty and colleagues have demonstrated that CDK6 causes resistance by inducing and binding INK4 proteins such as $p18^{INK4C}$. Notably, suppression of INK4 expression or its binding to CDK6 restores the sensitivity to CDK4/6i (83).

3.2. Inducing a senescence-like state

Inhibition of CDK4/6 has also been reported to activate a senescence-like state, normally featured by cellular enlargement and enhanced β-galactosidase activity (84) (85). The main phenotypic hallmarks of classical cellular senescence are irreversible cell cycle exit, apoptosis resistance, remodelling of chromatin, metabolic dysregulation, and secretion of cytokines/growth factors, known as senescence-associated secretory phenotype (SASP) (86). Watt *et al* (2021) have detected chromatin remodelling in CDK4/6i-treated BC cell-based models, mouse models, and clinical specimens, defined by widespread enhancer induction (87). Newly activated enhancer-mediated transcriptional activity is directly involved in regulating apoptotic evasion, increased cellular immunogenicity, and is mainly guided by activator protein 1 (AP-1) transcription factors (88). Particularly, these new enhancers also determine a stronger differentiation of cellular phenotype, emphasizing an association between Rb induction and cellular differentiation in tumors (89). However, this senescence-like state is in some cases reversible when CDK4/6 inhibition is removed, since cells re-enter cell cycle and restart cell proliferation, indicating that CDK4/6i do not, in this particular case, cause an irreversible cell cycle exit and a senescence state (90). CDK4/6i have shown induction of *in vitro*/*vivo* senescence in several tumor types, including HR^+ BC (84) (91). This senescent phenotype mostly depends on Rb (92), but may also be associated with decreased CDK4/6-mediated phosphorylation on DNA methyltransferase 1 (DNMT1) and forkhead box M1 (FOXM1) (93). Interestingly, suppression of mTOR complex 1 (mTORC1) during palbociclib treatment prevents the activation of senescence, whereas genetic depletion of mTORC1

negative regulator tuberous sclerosis complex 2 (TSC2) leads to sustained mTORC1 activity and induction of senescence (94). This is in line with recent publications showing a major role for active cellular growth signalling in CDK4/6i sensitivity during palbociclib treatment (95) (96) (97). Interestingly, treatment with palbociclib or abemaciclib can downregulate mTOR signaling in BC, supporting the notion that CDK4/6i can also prevent senescence through the downregulation of mTOR signaling in BC cells (94) (98). Notwithstanding p53 is a crucial player in classical senescence, its role in CDK4/6i-induced senescence has not been fully elucidated. Indeed, *TP53* mutations have recently arisen as the most valuable genomic predictor of CDK4/6i resistance in a panel of 560 tumor cell lines (99) (51), indicating an active contribution of functional p53 to drug-mediated proliferative repression. On the contrary, CDK4/6 inhibition has also been found to activate phenotypic aspects of senescence in cancer cells with unfunctional p53 (100) (101) (102). Since both p53 and Rb may overlap their diverse actions in the induction of typical senescence, p53 wild-type, as well p53 mutant BC cells, may possibly show aspects of senescence in response to CDK4/6 suppression; but qualitatively these two senescent phenotypes are distinct (51). Further studies are required to fully understand how CDK4/6i generates senescent phenotypes, such as SASP state, in HR^+ BC; and, better clarify what roles *TP53* plays in regulating CDK4/6i resistance.

3.3. Evading apoptosis

Even though CDK4/6i can activate a senescent-like state there are still doubts as to whether these agents are able to exert a direct killing of luminal HR^+ BC cells. Indeed, like senescent cells, BC cells that enter CDK4/6i-triggered senescence exhibit resistance to apoptotic insults (103) (87). Several studies have suggested that CDK4/6i inhibit apoptosis, simply by inducing senescence, which represents an anti-apoptotic state (80). For instance, co-treatment of palbociclib and ET reduces apoptosis in HR^+ BC cells (104). Thus, further clinical studies are required to consider using senolytic agents that can selectively kill BC senescent cells. Apoptosis resistance in BC partly derives from the induction of a super-enhancer spanning the *BCL2L1* gene, whose activity augments intra-tumoral levels of Bcl-xL, a notorious anti-apoptotic Bcl-2 family protein (105) (106). Accordingly, this apoptosis-resistance state can be reversed by Bcl-xL inhibitors (107), which re-establish susceptibility to apoptosis in CDK4/6i-pre-treated HR^+ BC cells (87). At present, anti-apoptotic BH3-family proteins are considered the principal molecules involved in CDK4/6i-mediated apoptotic evasion in HR^+ BC cells. Pre-clinical studies report that Bcl-2 inhibitor BH3-mimetic venetoclax (108) increases response to CDK4/6i palbociclib by triggering apoptosis in BC cells, including in senescent cells. In line with this, the primary objective of the ongoing PALVEN phase 1b clinical trial (NCT03900884), exploring the combination of CDK4/6i palbociclib, aromatase inhibitor (AI) letrozole, and venetoclax, is to determine the maximum tolerated dose (MTD) and the recommended phase 2 dose (RP2D) of this co-treatment therapy in HR^+ BC patients (109). Interestingly, the recent VERONICA phase 2 clinical trial (NCT03584009) did not indicate clinical utility of combining venetoclax and fulvestrant in ET-resistant, CDK4/6i-refractory metastatic $HR^+/HER2^-$ BC patients; but does suggest enhanced dependence on Bcl-xL in this setting. Indeed, there was prolonged PFS and a marginal improvement in clinical benefit rate (CBR) with venetoclax in cancers exhibiting strong Bcl-2 expression (IHC 3+) and a Bcl-2/Bcl-xL histoscore ratio ≥1, indicating dependence of cancer survival on Bcl-xL in the post-CDK4/6i setting (110). Further insight is required to fully clarify how CDK4/6i-mediated cancer regression occurs, and establish new strategic therapeutic combinations using CDK4/6i and newer anti-apoptotic inhibitors, such as venetoclax. Interestingly, despite these aforementioned insights, a certain number of studies address the possibility that CDK4/6i can directly determine apoptosis in ER^+ BC cells (20) (15) (80) (111). Indeed, prolonged

abemaciclib treatment can also trigger apoptosis in Rb-proficient BC cell lines, as indicated by TUNEL staining and annexin V staining (20). Accordingly, CDK4/6i-induced endorsement of apoptosis has also been detected in leukemic cells (112). Hence, abemaciclib treatment-mediated inhibition of BC proliferation can possibly occur through the promotion of long-term modifications in BC cells including apoptosis. These *in vitro* results suggest that short-term abemaciclib treatment inhibits BC cell progression, whereas long-term abemaciclib exposure can result in sustained anticancer effects through the activation of apoptosis, the induction of senescence, and the alteration of cellular metabolism (20).

3.4. Enhancing Autophagy

Recent studies suggest that further exploration is warranted focused upon how autophagy may decrease the efficacy of targeted therapies, ET, chemotherapy, radiotherapy, and immunotherapy *via* modulation of intermediate proteins. In fact, the potential use of autophagy inhibitors to improve the antitumor effects of agents by avoiding cyto-protective autophagy is an active focus area in cancer biology (113) (114) (115). Interestingly, regulation of senescence and autophagy often depends on the same signaling pathways (116) (117). Activity of the cyclin D-CDK4 axis is crucial for the suppression of autophagy in mammary epithelial cells (118). In fact, studies have demonstrated that pharmacological suppression of CDK4/6 signaling axis determines autophagy in both normal breast cells and BC cells (119) (90). In line with this, CDK4/6i enhance several markers of autophagy in HR^+ BC cell lines and xenograft models. A combination of autophagy inhibitors, such as hydroxychloroquine and chloroquine, and CDK4/6i, therefore displays a synergistic effect. This combinatorial treatment cannot kill CDK4/6i-treated BC cells, but instead, induces a permanent cell cycle exit via senescence *in vitro*/*in vivo* (90). Suppression of CDK4/6 and autophagy is also synergistic in other solid tumors with normal G1/S checkpoint, which may indicate promising new combination therapies (69).

3.5. Epigenetic regulation

Using cell-based and mouse models of BC, Watt *et al* have recently shown that CDK4/6i can reprogram the active enhancer landscape by stimulating the transcriptional activity of AP-1 in an Rb-dependent manner (120) (121) in luminal BC cells *in vitro*/*vivo* (87). Even though chromatin at the promoters of cell cycle genes displays CDK4/6i-induced suppressive modifications, numerous intergenic and intronic regions exhibit augmented accessibility to H3K27ac, a histone H3 epigenetic modification (122) associated with higher activation of transcription (87). Importantly, these CDK4/6i-induced enhancers, which are regulated by AP-1 transcription factor family members, play critical roles in resistance to apoptosis, luminal differentiation, and tumor immunogenicity. Therefore, inhibition of CDK4/6 increases the levels AP-1 transcription factor family members (87), which are in turn involved in the activity of these new enhancers (80). In line with this, AP-1 also guides enhancer activation and chromatin accessibility in benign senescent cells (88). More studies are required to interrogate whether the estrogen receptors itself, or its cofactor, the pioneer transcription factor FOXA1, is/are involved in CDK4/CDK6i-mediated induction of enhancers, and whether concurrent treatment of CDK4/6i with ET can reshape these chromatin-based mechanisms.

3.6. Interaction with oncogenic kinase signaling pathway

Combination of CDK4/6i and growth factor receptor, e.g. receptor tyrosine kinase (RTK) (123), or PI3K/AKT/mTOR pathway (124) (125) inhibitors, has shown synergistic or strengthened

effects (80). Triple combination of CDK4/6i, PI3Ki, and ET leads to strong efficacy *in vivo*, in term of rapid tumor growth reduction, in HR$^+$ BC models. In addition, continuous exposure of this triplet *in vitro* determines significant decrease of colonies, compared to the palbociclib + fulvestrant doublet, in MCF-7 and T47D cell lines. Besides this triplet combination displays higher reduction of pRb S807/811 phosphorylation, decreased cyclin E2 and CDK2 expression, and augmented poly (ADP-ribose) polymerase (PARP) cleavage, in comparison to the palbociclib + fulvestrant doublet, in both cell lines (126). The molecular mechanisms underlying the additive or synergistic effects of these beneficial preclinical combinations have not been fully elucidated. This synergism may be explained by the fact that these signaling pathways affect cell-cycle machinery through cyclin D-CDK4/6 (127). A typical hallmark is the augmented activity of upstream pathways, especially the PI3K/AKT/mTOR pathway (128), in CDK4/6i-treated luminal BC cells (125). In addition, CDK4/6i also enhance the phosphorylation of human epidermal growth factor receptor (HER) family and AKT in luminal HER2-positive (HER2$^+$) BC cell lines (129). These phenomena may be partially explained by cyclin D/CDK4 and cyclin D/CDK6 phosphorylation activities. In fact, without inhibition of CDK4/6, cyclin D/CDK4 and cyclin D/CDK6 can phosphorylate TSC2, which negatively regulates mTOR (98). Conversely, CDK4/6i-mediated suppression of CDK4/6 decreases TSC2 phosphorylation, thereby reducing the action of mTORC1, and rebounding upstream RTK activity (130) (98). A direct effect of enhanced RTK signaling is a continued induction of mTORC1 activity, which can lead to progression of S phase (131). Alternatively, augmented levels of cyclin D may contribute to forming anomalous cyclin D/CDK2 complexes that increase phosphorylation on Rb (126). As a result, new combinations of CDK4/6i and growth factor pathway inhibitors have reached clinical trials. In line with this, studies exploring the combination of CDK4/6i and HER2 inhibitors, as well as CDK4/6i and PI3K inhibitors, have been initiated (69). Regarding RTKs, combination of CDK4/6i abemaciclib, SERD fulvestrant, and monoclonal antibody trastuzumab has demonstrated improved PFS and safety profile when compared to standard-of-care (SOC) chemotherapy and trastuzumab in pretreated HR$^+$, HER2$^+$ advanced BC patients. Importantly, this clinical study indicates that a chemotherapy-free regimen can be a potential alternative therapy for these patients (132). In relation to PI3K/AKT/mTOR pathway (133), triple combination of CDK4/6i palbociclib with PI3Kαi taselisib and fulvestrant has exhibited an overall response rate (ORR) of 37.5%, and a favourable safety profile, in *PIK3CA*-mutant ER$^+$/HER2$^-$advanced BC patients (134).

3.7. Augmenting immunogenicity

CDK4/6i have been shown to improve anticancer immune responses in several preclinical studies of BC. Indeed, it has been demonstrated that CDK4/6i promote tumor microenvironment inflammation, and CD4$^+$ T-cells and/or CD8$^+$ T-cells can in part mediate therapeutic responses (103) (135) (136). This phenomenon is detected in all FDA-approved CDK4/6i. Inhibition of CDK4/6 augments antigen presentation on major histocompatibility complex (MHC) class I molecules, via Rb, in cancer cells (137). Interestingly, CDK4/6i decrease the expression of (the E2F target gene) DNA methyltransferase 1-encoding *DNMT1*, leading to hypomethylation, and thence transcription of endogenous retroviral elements (138). Consequently, double-stranded RNA in the cell activates a viral mimicry response, defined by generation of interferon, as well as expression of interferon-induced genes. In addition, chromatin remodeling, mediated by CDK4/6i, triggers activity of enhancers that overlie endogenous retroviral element sequences possibly involved in driving interferon-stimulated gene expression (80) (87). CDK4/6i also promote metabolic stress in BC cells, resulting in chemokine expression including CXCL10 and CCL5, which can further augment the anticancer immune response (139). Importantly, CDK4/6i exert direct effects on T-cells. Indeed, various

CDK4/6i can effectively repress, perhaps via Rb, regulatory T-cells (Tregs) proliferation in the tumor microenvironment (140) (141) (4). Conversely, CDK4/6i can enhance effector T-cell function, highlighted by decreased expression of T-cell exhaustion markers, and by increased production of effector cytokines. This is partially due to suppression of CDK6-induced phosphorylation of nuclear factor of activated T-cells (NFAT) transcription factor members (142) (135). In addition, CDK4/6i can also favour $CD8^+$ T-cell differentiation toward a memory cell fate, thereby contributing to increasing anticancer efficacy (143) (137). These phenomena lead to a T-cell-inflamed tumor microenvironment and augmentation of effector T-cell activity (135), resulting in enhanced anticancer effects of CDK4/6i (137). Accordingly, combination of different CDK4/6i and several immunotherapies have demonstrated more significant reduction in cancer growth, as well as improvement of T-cell memory, compared to each agent alone, in preclinical studies not including ER^+ BC animal models (80). In the recent RIBECCA phase 3 clinical trial (NCT03096847), ribociclib treatment has demonstrated the induction of an already-existing immune response instead of *de novo* immune activation in ER^+ BC patients (144). Gene expression analyses of biopsies suggests that this immune effect occurs in luminal BC patients. Indeed, in the neoMONARCH clinical trial, combination of CDK4/6i abemaciclib and aromatase-inhibitor anastrozole have shown significant efficacy via potent cell-cycle arrest and augmented immune activation, with manageable toxicity, in $HR^+/HER2^-$ early BC patients (145). However, leveraging these CDK4/6i-mediated immune effects to improve patient outcomes have encountered challenges related to: *1)* observations that combinations of CDK4/6i and immuno-oncology therapy have been hindered by unfavourable safety profiles; and *2)* ER^+ metastatic BC has not displayed satisfactory responses to immune-based strategies (80). Interestingly, in two neoadjuvant trials of CDK4/6i plus ET, highly-enriched interferon (IFN)-related signatures have been observed in ER^+ BC patients with intrinsic and acquired resistance to CDK4/6i, suggesting that aberrant IFN signaling is an important driver of resistance (146).

4. Available and emerging CDK4/6i in ER^+ breast cancer

Palbociclib (147) (148) (149), ribociclib (150) (151) (152), and abemaciclib (153) (154) (155) (156) have shown a statistically significant PFS benefit when used in combination with ET in first/second-line advanced $ER^+/HER2^-$ BC clinical settings. Despite similar improvements in PFS benefit, important differences have emerged with regard to overall survival in the first-line setting (157) (158) (159). Only clinical studies using ribociclib in co-administration with AI have demonstrated a significantly prolonged overall survival (OS) (160) (161). In fact, OS benefit has not been observed for first-line palbociclib in combination with letrozole (162), and results regarding OS for first-line abemaciclib in combination with AI (NCT02246621) have not been reported yet (163). Having demonstrated beneficial results in metastatic setting, CDK4/6i have also been tested in the adjuvant setting for early-stage $HR^+/HER2^-$ BC patients. The PALLAS (NCT02513394) and the Penelope-B (NCT01864746) clinical trials failed to display an invasive disease-free survival (IDFS) benefit by adding 1 year (164) and 2 years (165) of palbociclib, respectively, to adjuvant ET. Nevertheless, the phase 3 monarchE clinical trial (NCT03155997), demonstrated that adding 2 years of abemaciclib to adjuvant ET significantly improves IDFS in high-risk early-stage $ER^+/HER2^-$ BC patients. Indeed, abemaciclib decreases the risk of recurrence, and its benefit appears to extend beyond the completion of treatment, with an absolute increase at 4 years (166). Besides, data from the ongoing phase 3 NATALEE clinical trial (NCT03701334), which is evaluating the broadest population to date of $HR^+/HER2^-$ early non-metastatic BC patients treated with 3 years of ribociclib and ET, are still pending. Notably, the reduced starting dose of ribociclib in this trial is expected to further minimize dose-dependent toxicities without compromising the efficacy of the co-treatment.

Thus, the NATALEE clinical trial will plausibly provide important answers that at present are still unmet (167). The contrasting results between the PALLAS/Penelope-B clinical studies and the monarchE trial are possibly due to duration of the CDK4/6i treatment, pharmacological distinctions between the agents, and differences in study population. Additional CDK4/6i are being investigated in clinical trials. In the DAWNA-2 phase 3 clinical trial (NCT03966898), combination of CDK4/6i dalpiciclib and anastrozole or letrozole, has shown a significantly longer PFS (30.6 months) compared to placebo plus letrozole or anastrozole (18.2 months), suggesting that these co-treatments could be a novel alternative first-line treatment alternative option to the current treatment landscape, in $HR^+/HER2^-$ advanced BC patients. Grade 3 or grade 4 adverse events were seen in 90% of patients with dalpiciclib and 12% with placebo, whereas serious adverse events were 12% in dalpiciclib-treated patients and 7% in placebo-exposed patients (27). Other clinical trials are testing the anticancer activity and safety of novel CDK4/6i in $HR^+/HER2^-$ advanced BC patients. Indeed, a phase 3 clinical trial (NCT05077449) is investigating birociclib in combination with fulvestrant; a phase 3 clinical trial (NCT05438810) is evaluating FCN-437c in combination with fluvestrant ± goseraline; phase 3 clinical trials are using TQB3616 combined with ET (letrozole or anastrozole or tamoxifen) (NCT05780567) and fulvestrant (NCT05375461); a phase 2 clinical trial (NCT03519178) is investigating CDK2/CDK4/CDK6 inhibitor PF-06873600 either alone or in combination with hormone therapy; a phase 2 clinical trial (NCT05085002) is evaluating CDK4/CDK6/CDK9 inhibitor lerociclib in combination with standard ET (fulvestrant or letrozole); a phase 2 clinical trial (NCT04282031) is using BPI-1178 in combination with fulvestrant or letrozole; and finally, a phase 1 clinical trial (NCT04433494) is testing TY-302 both singly and in combination with tamoxifen. The clinical trials outlined above, including those that are ongoing, have established the following points, and may provide further insights related to: *1)* the role CDK4/6i monotherapy treatment can play in mediating antiproliferative and clinical anticancer activity in both pre- and post-menopausal HR^+ BC patients; *2)* how co-treatment with CDK4/6i and ET synergistically improves clinical outcomes, suggesting that CDK4/6 inhibition can delay or overcome ET resistance; and *3)* how different toxicity profiles of CDK4/6i impact their treatment schedule, leading in some cases to intermittent dose interruptions as opposed to continuous administration, for example as it necessary with regard to palbociclib/ribociclib versus abemaciclib (51) (**FIGURE 4**) (**SUPPLEMENTARY FIGURE 1**) (**TABLE 1**) (**SUPPLEMENTARY TABLE 1**)

5. Mechanisms of resistance to CDK4/6i in ER^+ breast cancer

Though small-molecule CDK4/6i have shown significant clinical benefit, treatment failure and eventual resistance still represent major obstacles for HR^+ BC patients (148) (168) (169). In the PALOMA-3 clinical trial, analysis of circulating tumor DNA (ctDNA) from HR^+ BC patients demonstrated that two different types of resistance can occur due to co-treatment of CDK4/6i and anti-estrogens. In this study, mutation-induced resistance depended on the type of treatment and appeared at definite times. This suggested that mutations causing initial resistance are different compared to mutations needed for resistance arising after a period of efficacious treatment (170). Hence, resistance occurring immediately after treatment that cause no response to BC patients is referred as intrinsic resistance (171), whereas resistance occurring in BC patients who initially respond but progress later on treatment is defined as acquired resistance (28). Estrogen receptor (ER) is the only routinely utilized clinical biomarker to select ER^+ BC patients for co-treatment of CDK4/6i and ET (172) (170). Numerous preclinical studies have implicated a wide array of potential resistance mechanisms (**FIGURE 5**), such as enhanced activity through oncogenic signaling pathways, modifications

of cell cycle machinery components, metabolic variations within tumor cells, and drug-induced alterations in stromal function (9). In line with this, several mechanisms of CDK4/6i resistance highlight new targets, such as signaling mediators AKT1, fibroblast growth factor receptor (FGFR), epidermal growth factor receptor (EGFR), and RAS, as well as cell cycle elements, including cyclin E, CDK2, CDK6, Rb1, AURKA, and c-Myc (173) (174). It is possible that suppression of CDK4/6 can delay initiation of endocrine resistance, and resistance to combination therapy is mainly caused by ET backbone-associated resistance (28). In favor of this, biomarker analysis of ctDNA in MONARCH-3 clinical trial has demonstrated reduced frequency of *ESR1* mutations with the combination of abemaciclib and AI (17%) in comparison to AI singly (31%) (175). In this section, the genomic aberrations identified to date, and the major mechanisms of resistance presently sustained by preclinical studies and clinical trials are discussed in detail (**FIGURE 5**).

5.1. Loss of Rb function

CDK4/6i act to preclude CDK4/6-mediated phosphorylation-induced Rb protein inactivation (176) (80). Thus, mutations leading to *RB1* biallelic loss of function drive resistance to CDK4/6i (126). Accordingly, cells harboring a dysfunctional Rb protein display continuing proliferation, and their cell cycle remains unchecked even during exposure to CDK4/6i. Therefore, loss of functional Rb is a well-established mechanism of resistance to CDK4/6i, though it appears to occur only rarely. Indeed, combinations of palbociclib and fulvestrant, or ribociclib and letrozole, have been shown to provoke *RB1* mutations in metastatic ER$^+$ BC patients (177). In the PALOMA-3 phase 3 clinical trial (NCT01942135), using whole-exome sequencing of paired ctDNA samples, acquired *RB1* mutations were seen in BC patients who progressed on the combination of palbociclib and fulvestrant (28). Moreover, combination of ribociclib and ET displayed shorter PFS in cancer patients harboring *RB1* mutations compared to wildtype (178). Notoriously, *RB1* loss of heterozygosity is also related to intrinsic CDK4/6i resistance. In addition, *RB1* mutations are commonly acquired in CDK4/6i-treated ER$^+$ BC patient-derived xenografts and ER$^+$ BC patients with pre-existing *RB1* loss of heterozygosity (179). Other clinical studies have also identified loss of functional *RB1* as a principal feature of resistance to CDK4/6i in HR$^+$ metastatic BC (173) (**FIGURE 5**).

5.2. Acquired CDK6 amplification

Interestingly, CDK6 amplification can also provoke BC resistance to CDK4/6i (180). It is unclear whether this phenomenon is due to incomplete drug-mediated CDK6 inhibition, or alternatively, to other kinase-independent effects of CDK6 (77). CDK6 upregulation can occur in response to continued exposure to CDK4/6i, and sensitivity can be re-established via subsequent CDK6 knockdown in preclinical studies. Accordingly, a recent clinical study has shown an inverse correlation between CDK6 amplification and shorter PFS with CDK4/6i treatment in ER$^+$ BC patients. Besides, combined score of cyclin E1, p-CDK2, and CDK6 predict a worsen outcome in co-treated ER$^+$ BC patients, as well as in only ET-treated ER$^+$ BC patients (181). Cadherin superfamily member FAT1 (182) loss of function mutations, though rare, are related to CDK4/6i resistance, possibly by enhancing the expression of CDK6 in ER$^+$ BC patients (183). Notably, FAT1 inactivation/deletion leads to induction of Hippo signal transduction pathway (184), known to regulate apoptosis and cell growth (185). Consequently, loss of FAT1 promotes augmentation of Yap/Taz transcription factors, whose activity endorses the overexpression of CDK6. Patients with biallelic FAT1 inactivation demonstrate a 2.4-month PFS, compared to ER$^+$ BC patients harboring FAT1 missense mutations, that show only a slightly shorter PFS (10.1 months) when compared to patients with wildtype FAT1 (11.3

months) (183). The exosomal miRNA-432-5p, which is a target of TGFBR3 and SMAD4, also confers acquired resistance to CDK4/6i by augmenting CDK6 expression, downregulating SMAD4, and ultimately reducing G1/S cell cycle arrest (186). Interestingly, removal of palbociclib downregulates CDK6, cyclin D1-encoding *CCND1*, and miRNA-432-5p; and conversely, upregulates Rb. Thus, resistance of ER^+ BC cells to palbociclib can be modulated both *in vitro* and preclinical models (186), which may suggest why some ER^+ BC patients progressing on a particular CDK4/CDK6i may subsequently respond to a distinct CDK4/CDK6i (**FIGURE 5**).

5.3. Abnormal cyclin E/CDK2 activity

Evasion from CDK4/6i may also occur via Rb phosphorylation through upregulation of cyclin E/CDK2. Indeed, cyclin E1 and cyclin E2 amplification leads to augmented CDK2 activity and decreased expression of p27 (187) (176) (80). Cyclin E2-encoding *CCNE2* gene amplification is often detected in HR^+ metastatic BC treatment-resistant tumor specimens (173), and overexpression of G1/S-specific cyclin-E1-encoding *CCNE1* mRNA is associated with poor response to palbociclib in tumor tissue taken from HR^+ metastatic BC patients (188). This may be due to the fact that elevated levels of cyclin E results in CDK2-induced phosphorylation of Rb, overcoming CDK4/6i-generated G1-phase arrest (126). CDK2 mRNA expression is also upregulated in ER^+ cell lines resistant to palbociclib; and susceptibility to palbociclib can be successfully re-established by siRNA-mediated CDK2 knockdown, leading to enhanced apoptosis and senescence (189) (9). Interestingly, mesenchymal-epithelial transition factor (c-MET) (190) signaling, and its downstream effector focal adhesion kinase (FAK) (191), may also contribute to abnormal CDK2 activation independently of CDK4/6 (192). Accordingly, ctDNA analysis of abemaciclib monotherapy has shown 8% acquired genomic aberrations in *MET* (175). Moreover, PI3K/AKT/mTOR pathway upregulation can also promote non-canonical CDK2 activity by binding to cyclin D, leading to consequent progression of cell cycle and acquired resistance to CDK4/6i (126) (**FIGURE 5**).

5.4. Oncogene c-Myc alteration

Members of the MYC oncogenic family are transcription factors responsible for the regulation of several genes. c-Myc gene, which belongs to the MYC family, is often persistently expressed in cancer (193), leading to enhanced expression of various genes involved in cell proliferation (194). In addition, c-Myc has been found to be highly expressed in BC (195) (194). c-Myc is activated by CDK2, CDK4 and CDK6, and is upregulated in preclinical models of CDK4/6i resistance (189) (196). In line with this, the nextMONARCH-1 clinical trial (NCT02747004) has shown an enhancement of acquired *MYC* genomic aberrations after treatment of abemaciclib monotherapy or in combination with a nonsteroidal AI, in HR^+ BC patients (175). Moreover, S6K1 kinase activation has been detected in more than 10% of BC patients, and several studies have shown that increased S6K1 can drive palbociclib resistance by inducing c-Myc signal transduction in preclinical models and clinical ER^+ BC samples (197) (**FIGURE 5**).

5.5. Activation of growth factor signaling pathways

Analysis of genomic alterations in clinical samples has implicated growth factor signal transduction pathways as a mechanism of CDK4/6i resistance. Determining the unilateral impact of growth factor pathway activation on CDK4/6i resistance is complex, as most patients are treated with the combination of these agents and ET. Many of these pathways have been involved in ET resistance. Collecting tumor biopsies at progression on CDK4/6i in several

cohorts have provided further insights related to acquired and intrinsic resistance. These studies have demonstrated significant enrichment of hyperactivating alterations in *ERBB2*, *PTEN*, *AKT1*, *FGFR*, *KRAS*, *HRAS*, and *NRAS* genes, in CDK4/6i resistant cancers (80) (173). Additionally, similar findings have been discovered in ctDNA biomarker analysis performed in the MONARCH-3 clinical trial (NCT02246621), by which acquired mutations in *FGFR1* and *EGFR* and are detected in the abemaciclib group (175). Elevated phospho-AKT level in BC metastases is also biomarker for poor prognosis and is associated with reduced PFS in ER^+ BC patients who have been administered CDK4/6i and ET (198). Nonetheless, these analyses are limited by multiple factors, including the absence of control group consistent of an ET-only treated cohort, administration of other lines of therapy after CDK4/6i before acquiring cancer tissue for assessment, and a modest number of BC patients. These studies suggest that alterations in growth factor signaling pathways, especially the PI3K/AKT/mTOR axis (133), can contribute to CDK4/6i resistance. However, the mechanism of action whereby these mutations confer CDK4/6i resistance, and their relative contribution to concurrent ET resistance, is still uncertain (199) (**FIGURE 5**). Considering recent evidence that active cellular growth signalling promotes CDK4/6i sensitivity (95) (96) (97) these mechanisms of resistance might be specific for the combinatorial treatment of CDK4/6i with ET. The activation of the MAPK signaling pathway and its key effectors, RAS, RAF, MEK, and ERK, has also been observed in BC preclinical models that are resistant to CDK4/6i. Notably, the triple combination of MEK inhibitors, CDK4/6i and ET has shown promising anticancer activity in CDK4/6i-resistant models (200) (201). These findings suggest that MEK inhibition may be a novel valuable therapeutic option to treat or prevent CDK4/6i resistance. Besides, the loss of neurofibromin 1 (NF1), a gene that downregulates RAS signaling and cellular proliferation, is an established mechanisms of resistant to ET (202). However, its role in CDK4/6 resistance remains to be fully clarified.

5.6. CDK4/6i-induced rewiring of the PI3K/AKT/mTOR pathway

In the PI3K/AKT/mTOR signaling pathway, activation of PI3K mainly occurs by growth factor-induced receptors, such as receptor tyrosine kinases (RTKs). PI3K is then recruited to its substrate PIP2, endorsing the production of PIP3. Inactive AKT in the cytoplasm binds to PIP3 on the cell membrane and allows phosphorylation by PDK1 and mTORC2, resulting in complete activation of AKT, which successively phosphorylates multiple sites on the tuberous sclerosis complex 2 (TSC2). The TSC2 protein forms a functional complex with tuberous sclerosis complex (TSC1) protein called TSC1-TSC2 complex or simply tuberous sclerosis complex (TSC). AKT-mediated phosphorylation on TSC2 hinders the ability of TSC to act as a GTPase-activating protein (GAP) toward the small GTPase Rheb, endorsing Rheb-GTP accumulation (203). Importantly, the PI3K/AKT/mTOR axis is regulated by negative feedback, via S6K/IRS/GRB10, and positive feedback, via IKKα/NFκB/PTEN, to ensure that signal transductions are captured and conveyed transiently (133). Importantly, suppression of CDK4/6 can promote adaptive rewiring of the PI3K/AKT/mTOR signaling pathway in BC cells, thereby augmenting their dependence on this axis. Indeed, inhibition of CDK4/6 has been found to enhance phosphorylation and activity of HER receptors and PI3K/AKT/mTOR pathway in various HR^+ BC models (51). This adaptive rewiring is driven by three diverse mechanisms: *1)* CDK4/6 directly activates mTORC1 through phosphorylation of the tumour suppressor TSC, and partial inhibition of mTORC1 mediated by CDK4/6i can relieve feedback suppression on upstream RTKs (98) (129); *2)* CDK4/6i-mediated hypo-phosphorylation of Rb directly promotes access of AKT to stress-activated protein kinase-interacting protein (SIN1), a crucial component of mTOR complex 2 (mTORC2), leading to increased mTORC2-induced phosphorylation of AKT (204); and *3)* CDK4/6i activate secretion of cancer cell-derived

growth factors, perhaps as part of SASP, which trigger signaling through RTKs in an autocrine manner (205). These adaptive changes may dictate acquired CDK4/6i resistance, and cause failure of CDK4/6i monotherapy, emphasizing the importance of combinatorial studies targeting CDK4/6 and PI3K/AKT/mTOR pathway or other mitogenic signaling axes. Notably, upstream mitogenic pathways augment CDK2 activity, endorsing Rb phosphorylation and S phase entry, in spite of ongoing CDK4/6 inhibition (126) (206) (207). Mitogenic signaling-mediated enhancement of CDK2 activity may occur through p21/p27 sequestration in cyclin D1-CDK4/6 complexes, direct downregulation of p27, and increased formation of atypical cyclin D1/CDK2 complexes caused by higher levels of cyclin D1 (51). Besides, enhanced mitogenic signalling pathways augments mTORC1 activity, which regulates S phase entry and drives proliferation despite continued blockage of CDK4/6 (208) (51). Accordingly, numerous studies have shown synergistic efficacy by co-inhibiting CDK4/6 and mTOR (209). Additionally, in the phase 1b TAKTIC clinical trial (NCT03959891), combination therapy with CDK4/6i, AKT inhibitor ipatasertib, and ET, has shown significant clinical activity, emphasizing the importance of PI3K/AKT/mTOR pathway inhibitor-based combinations in HR^+/HER^- metastatic BC patients (210). BC cell responses to co-treatment of CDK4/6i and PI3K/AKT/mTOR inhibitor include cytostasis and/or apoptosis. Normally, combination-induced cytostasis is characterized by increased Rb hypo-phosphorylation compared to either inhibitor alone, followed by enhanced downregulation of E2F targets and augmented senescence (211) (129) (126) (212) (51) (**FIGURE 6**).

5.7. Proliferation mechanisms despite CDK suppression

Due to the intrinsic plasticity of cell cycle machinery, BC cells may develop peculiar mechanisms whereby they can still divide in spite of co-inhibition of all CDKs acting in the interphase. It was believed that mammalian cells required the subsequent induction of CDK2, CDK3, CDK4 and CDK6, to guide cells through interphase, and CDK1 activation to advance during mitosis (213). Nonetheless, recent genetic data have shown survival of mice in the absence of different CDKs. Indeed, mammalian cells absent of CDK2, CDK3, CDK4, and CDK6 are still able to proliferate via CDK1. Thus, it would be important to investigate whether BC cells exposed to combinations of CDK2 inhibitor (CDK2i) and CDK4/6i may also adopt this particular mechanism (214). Interestingly, while cells treated with the specific CDK2 inhibitor PF3600 (196) (215) can proliferate by potentially using CDK1, combinatorial treatment with CDK2i and CDK4/6i prevents this phenomenon (216). Overall, this plausible mechanism could be overcome by suppressing CDK7, which exerts two essential roles: *1)* as CDK-activating kinase, exerting phosphorylation on CDK1, CDK2, CDK4 and CDK6; and *2)* as an intermediary molecule of RNA polymerase II-induced transcription (217) (218). Accordingly, specific CDK7 inhibitors such as samuraciclib have been evaluated in clinical development (NCT03363893) and have displayed promising antitumor activity in CDK4/6i-resistant $HR^+/HER2^-$ advanced BC patients (219). Furthermore, recent work from Wilson *et al* (2023) has shown that cellular growth also has a central role in CDK7 inhibitor samuraciclib sensitivity. Indeed, increased growth alone determines sensitivity to a CDK7 inhibitor, contributing to explaining why some tumors are more susceptible to CDK inhibition than normally growing cells (220). Since enhanced growth signalling is common in cancer cells, these aforesaid studies help explain the anti-cancer efficacy of CDK inhibitors, but also suggests that decreased growth signalling might allow proliferation despite suppression of all CDKs.

6. Therapeutic strategies to address drug resistance following CDK4/6i treatment in ER^+ breast cancer

There are a variety of available and emerging therapeutic approaches that may be utilized after progression on combination of small-molecule CDK4/6i and ET, including: *1)* prolonging CDK4/6i after progression; *2)* targeting CDK2 concurrently with or following CDK4/6i resistance; *3)* co-targeting CDK4/6 and the PI3K/AKT/mTOR pathway; *4)* using new endocrine therapies; *5)* using non-endocrine therapies. Results from completed and current clinical trials in ER$^+$/HER2$^-$ BC patients following progression on CDK4/6i, are summarized in **SUPPLEMENTARY TABLE 2**; whereas ongoing clinical trials in ER$^+$/HER2$^-$ BC patients following progression on CDK4/6i, whose results are still being awated, are summarized in **SUPPLEMENTARY TABLE 3**.

6.1. Continuation of CDK4/6i blockade after progression

The potential utility of CDK4/6i after prior progression on one of these agents remains an important clinical question and an active area of research. In the phase 2 PACE trial (NCT03147287), the combination of fulvestrant plus palbociclib demonstrated no significant improvement in PFS (4.6 months) compared to fulvestrant alone (4.8 months), in advanced ER$^+$/HER2$^-$ BC patients who received prior CDK4/6i (91% of whom received palbociclib) and an AI (221). The triplet combination therapy with abemaciclib, programmed death-ligand 1 (PD-L1)-neutralizing antibody atezolizumab, and fulvestrant is being evaluated in the MORPHEUS HR$^+$ BC platform trial (NCT03280563) (222). In contrast to the results from PACE, in the phase 2 MAINTAIN trial (NCT05207709), the combination of altered ET backbone (fulvestrant or exemestane) and ribociclib exhibited significant PFS benefit (5.2 months) compared to a change in the ET backbone (fulvestrant or exemestane) plus placebo (2.7 months), in advanced ER$^+$/HER2$^-$ BC patients who progressed on a CDK4/6i (84% of whom received palbociclib). In this population the PFS rate was 41.2% at 6 months and 24.6% at 12 months with ribociclib, compared to 23.9% at 6 months and 7.4% at 12 months with placebo (223). In the phase 2 BioPER trial (NCT03184090), a change in the ET and continued palbociclib displayed a PFS of 2.6 months and a CBR of 34%, in advanced ER$^+$/HER2$^-$ BC patients who demonstrated prior clinical benefit with palbociclib plus ET. Notably, a biomarker signature of high cyclin E1 (≥ 10% BC cells with positive nuclear staining), low Rb score (< 1% BC cells with positive nuclear staining), and *ESR1* mutation was independently related to shorter PFS in this study, suggesting that there may be practical strategies to identify a subset of patients who may not obtain benefit from palbociclib treatment continuation (224). Moreover, in the phase 1b TAKTIC clinical trial (NCT03959891), the co-administration of palbociclib, AKT inhibitor ipatasertib, and ET determined a CBR of 48%, a PFS of 5.5 months, and am OS of 24.5 months, after prior CDK4/6i progression, in HR$^+$/HER$^-$ metastatic BC patients (210).

6.2. Targeting CDK2 concurrently with or after CDK4/6i resistance

Due to the important role of cyclin E/CDK2 in CDK4/6i resistance (189) (181), there has been increasing interest in targeting CDK2 either concurrently with or after occurrence of CDK4/6i resistance. Interestingly, the triple combination of non-selective CDK2i dinaciclib, CDK4/6i palbociclib and AI letrozole, demonstrated enhanced therapeutic efficacy compared to palbociclib and/or letrozole singly in preclinical studies (181). A variety of CDK2i are being investigated in phase 1/phase 2 clinical trials in ER$^+$/HER2$^-$ BC patients (NCT04553133 and NCT05252416), as well as advanced solid tumor patients (NCT05867251, NCT04553133 and NCT05252416). Indeed, in the recent VELA phase 1 clinical trial (NCT05252416), CDK2i BLU-222 monotherapy has shown preliminary evidence of cell cycle pathway inhibition, clinical

anticancer activity, and an acceptable safety profile, in HR+/HER2- BC and other advanced solid tumor patients. Dose escalation of BLU-222 is ongoing to reach the RP2D (225). In another recent phase 1/phase 2 clinical trial (NCT04553133), the novel CDK2i PF-07104091 has exhibited anticancer activity as a single agent [partial response (PR): 18.8 %, duration of response (DOR) > 6 months: 12.5%, stable disease (SD): 37.5%, and disease control rate (DCR): 61.5%], and a favorable safety profile in heavily-pretreated HR+/HER2- advanced/metastatic BC patients who progressed on prior ET plus CDK4/6i. Dose expansions of PF-07104091 are ongoing in combination with fulvestrant in HR+/HER2- BC patients (226). In addition, an ongoing phase 1/phase 2 clinical trial (NCT03519178) is evaluating the safety, tolerability, as well as pharmacokinetic and pharmacodynamic properties of the CDK2/CDK4/CDK6 inhibitor PF-06873600 both singly and in combination with ET (letrozole or fulvestrant) in heavily pretreated advanced/metastatic HR+/HER2- BC patients after progression on previous CDK4/6i, ET, and ≤2 prior lines of chemotherapy. Early results suggest that the combination of PF-06873600 and ET exerts promising preliminary anticancer activity, modulation of cell cycle pharmacodynamic biomarkers (Ki-67 and pRb) in paired cancer biopsies, and a manageable safety profile (227).

6.3. Co-targeting PI3K/AKT/mTOR pathway

FDA-approved small molecule inhibitors targeting the PI3K/AKT/mTOR axis are available and under clinical development. Due to the clinical advantage of prolonged CDK4/6 inhibition and the complex crosstalk between CDK4/6 and the PI3K/AKT/mTOR pathway, various combinations of PI3K/AKT/mTOR inhibitors are being investigated in ER+ BC patients who have displayed progression on CDK4/6i. Anticancer potency of PI3K/AKT/mTOR pathway inhibitors, as well as potential toxicity has prompted careful ongoing evaluation before establishing feasible multidrug regimens.

6.3.1. PI3K co-inhibition

Induction of PI3K/AKT/mTOR pathway activation via *PIK3CA* mutations frequently occurs in ER+/HER2- advanced BC patients and is associated with poor prognosis. In the phase 3 SOLAR-1 trial (NCT02437318), the combination of isoform-specific PI3Kα inhibitor alpelisib and fulvestrant demonstrated improvement in OS (by 7.9 months) compared to fulvestrant alone in *PIK3CA*-mutated advanced ER+/HER2- BC patients who had relapsed after previous AI. Even though the analysis of this trial did not reach statistical significance, the 7.9-month improvement in median OS upon alpelisib addition to fulvestrant treatment further supported the statistically significant prolongation of PFS detected with alpelisib + fulvestrant in this ER+/HER2- BC patient population, which presents a poor prognosis due to a *PIK3CA* mutation. Hence, this study established a new standard of care for patients with *PIK3CA* mutations following progression on first-line therapy, and alpelisib is now in widespread clinical use based upon these results. The safety profile was manageable, with relatively high rates of hyperglycemia, and ER+/HER2- BC patients who discontinued alpelisib and placebo due to adverse events were 25% and 4.2%, respectively (228). In the phase 2 BYLieve trial (NCT03056755), the co-treatment of the isoform-specific PI3Kα inhibitor alpelisib and fulvestrant improved PFS and OS with manageable toxicity in ER+ BC patients following progression on CDK4/6i with detectable *PIK3CA* mutations, as compared to SOLAR1 trial, which included relatively few patients with prior CDK4/6 exposure. Notably, the most frequent ≥ grade 3 adverse events were hyperglycaemia (28%) and rash (9%). Serious adverse events occurred in 26% of ER+ BC patients, and no treatment-related deaths were reported (229). In addition, there are a number of ongoing clinical studies evaluating co-administration of

alpelisib, along with other novel next-generation PI3K inhibitors and antiestrogen therapy in ER$^+$/HER2$^-$ BC patients following progression on CDK4/6i, including the SEQUEL-Breast phase 2 (NCT05392608) (230), and the EPIK-B5 phase 3 (NCT05038735) (231).

6.3.2. AKT co-inhibition

Analysis of clinical samples following CDK4/6 progression have implicated enhanced *AKT* signaling as a potential resistance mechanism (173). As a result, there has been increasing interest in the use of AKT-specific kinase inhibitors following CDK4/6 progression. Co-treatment with CDK4/6i palbociclib, AKT1/AKT2/AKT3 inhibitor capivarsertib, and fulvestrant, has demonstrated effectiveness in inhibiting cancer growth in preclinical models resistant to CDK4/6i and ET (198). Indeed, in the phase 1b TAKTIC clinical trial (NCT03959891), the combination of palbociclib, AKT inhibitor ipatasertib, and ET showed 48% CBR, 5.5 months PFS, and 24.5 months OS, after prior CDK4/6i progression, in HR$^+$/HER$^-$ metastatic BC patients (210). In line with this, the FAKTION phase 2 clinical trial (NCT01992952) interrogated the combination of capiversertib and fulvestrant and demonstrated superior PFS and OS compared to fulvestrant and placebo, in (CDK4/6i naïve) endocrine-resistant advanced ER$^+$/HER2$^-$ BC patients. Of note, clinical antitumor activity was more pronounced in PI3K/AKT/PTEN pathway-altered ER$^+$/HER2$^-$ BC patients (232). The phase 3 CAPItello-291 trial (NCT04305496) also explored the co-administration of capiversertib and fulvestrant and demonstrated significant improvement in PFS (7.2 months) compared to fulvestrant and placebo (3.6 months) in ER$^+$/HER2$^-$ BC patients, among whom 69% had received prior CDK4/6i therapy. The most frequent ≥ grade 3 adverse events after treatment with capivasertib plus fulvestrant were rash (12.1%) and diarrhea (9.3%) compared to patients receiving placebo (0.3% rash and 0.3% diarrhea, respectively). Adverse events resulting in discontinuation were observed in 13% of capivasertib-receiving patients and in 2.3% of placebo-receiving patients (233). Finally, the ongoing FINER phase 3 clinical trial (NCT04650581) is investigating the combination of AKT inhibitor ipatasertib and fulvestrant, in ER$^+$/HER2$^-$ BC patients who had progression on CDK4/CDK6i and ET.

6.3.3. mTOR co-inhibition

At present, co-treatment with exemestane and the allosteric non-competitive mTOR inhibitor everolimus has only shown limited clinical benefit in ER$^+$ BC patients after progression on CDK4/6i (234). In the TRINITI-1 phase 1/phase 2 clinical trial (NCT02732119), the triplet combination of ribociclib, exemestane, and everolimus, exhibited a PFS of 5.7 months, a CBR (at week 24) of 41%, and no detection of new safety signals, in heavily-pretreated advanced ER$^+$/HER2$^-$ BC patients after progression on CDK4/6i (235). In the successive B2151009 phase 1b clinical trial (NCT02684032), the combination of CDK4/6i palbociclib, ET, and the pan-PI3K/mTOR inhibitor gedatolisib displayed a 1-year PFS of 53% in metastatic ER$^+$/HER2$^-$ BC patients who had received prior CDK4/6i therapy (236). In addition, two ongoing clinical trials of triplet co-treatment with CDK4/6i, ET, and PI3K/AKT/mTOR pathway inhibitors include the CAPItello-292 phase 1b/phase 3 (NCT04862663) (237), and the VIKTORIA-1 phase 3 (NCT05501886) (238) trials.

6.4. Using new endocrine therapies

The development of novel antiestrogen therapies that have the potential to restore sensitivity after progression on standard hormonal agents remains an active and exciting area of research and clinical development (239). Four completed randomized clinical trials of next-generation

oral SERDs have shown conflicting results in advanced ER$^+$/HER2$^-$ BC patients who had progression on previous ET with/without CDK4/6i. In both the phase 2 SERENA-2 trial (NCT04214288) of camizestrant *versus* fulvestrant (240), and the phase 3 EMERALD trial (NCT03778931) of elacestrant *versus* physician's choice ET (241), SERD administration demonstrated meaningful PFS benefit. Conversely, the acelERA phase 2 clinical trial (NCT04576455) of giredestrant (242), and the AMEERA-3 phase 2 clinical trial (NCT04059484) of amcenestrant (243), failed to demonstrate any difference in PFS between SERD treatment and physician's choice ET. Notably, among these SERD monotherapy-based clinical studies, the EMERALD trial is the only study to mandate prior CDK4/6i. Importantly, there was also heterogeneity amongst these studies in the proportion of ER$^+$/HER2$^-$ patients with *ESR1* mutant disease. Across these clinical studies, the *ESR1* mutant subgroup demonstrated more clinical benefit in comparison to *ESR1* wildtype, further implicating ESR1 as a valuable biomarker for ER dependence in these patients (244). Based upon data from the EMERALD study, elacestrant was FDA approved for patient with metastatic HR$^+$ BC and *ESR1* mutations. For further reading, SERDs have been extensively reviewed by Downton *et al* (2022) (245). In additional to oral SERDs, there are a variety of additional antiestrogen agents under active clinical development, such as selective human ER partial agonists (ShERPA) TTC-352 (246), selective ER covalent antagonists (SERCA) H3B 6545 (247), complete ER antagonist (CERAN) OP-1250 (248), proteolysis targeting chimera (PROTAC) ARV-471 (249), and the third-generation selective ER modulator (SERM) lasofoxifene (250). Interestingly, preclinical results also support androgen receptor (AR) activation as a potential therapeutic approach for CDK4/6i and ET resistant ER$^+$ BC models (251). Indeed, induction of AR leads to abnormal ER chromatin binding distribution and alters crucial co-activators, such as p300 and SRC-3, thereby suppressing ER-transcribed cell cycle genes (252). Consequently, clinical trials have been investigating the selective androgen receptor modulator (SARM) enobosarm either singly (NCT04869943), and in co-treatment with abemaciclib (NCT05065411), in ER$^+$/HER2$^-$ metastatic BC patients who have progressed on CDK4/6i and ET.

6.5. Using non-endocrine therapies including antibody-drug conjugates

Chemotherapy can be a valuable option for ER$^+$ BC patients who have progressed on combination of CDK4/6i and ET. Novel classes of ETs can successfully delay the need for chemotherapy; however this approach is only possible in ER$^+$ BC patients who maintain dependence on ER signaling. Antibody drug conjugates (ADCs), which have immune-mediated and cytotoxic properties (253) (254), are a prominent class of therapies that have recently been deployed in ER$^+$ BC patients (255). Indeed, ADCs span the gap between cytotoxic drugs and monoclonal antibodies employed to increase the therapeutic efficacy of BC treatments (256) (257). Biochemically, ADCs are composed of tumor antigen-targeting antibodies linked to robust chemotherapy payloads. After directing cytotoxicity agents towards BC cells, the cleavable linker determines the extent of bystander killing of nearby cancer cells that may not express the target antigen (258). The HER2-directed ADC trastuzumab deruxtecan is formed by humanized anti-HER2 monoclonal antibody bound to a topoisomerase I inhibitor payload through a tetrapeptide cleavable linker. Interestingly, in the DESTINY-Breast03 phase 3 clinical trial (NCT03529110), trastuzumab deruxtecan demonstrated reduced risk of disease progression or death with an ORR of 79.7% compared to trastuzumab emtansine (34.2%), in advanced HER2$^+$ metastatic BC patients previously treated with trastuzumab and a taxane. Grade 3-4 drug-related adverse events were 45.1% with trastuzumab deruxtecan and 39.8% with trastuzumab emtansine. Based on these positive results in HER2$^+$ metastatic BC patients treated with a prior anti-HER2-based regimen, trastuzumab deruxtecan has been FDA-approved for use in this cohort (259). In the DESTINY-

Breast04 phase 3 clinical study (NCT03734029), trastuzumab deruxtecan resulted in significantly longer PFS (10.1 months) compared to treatment of physician's choice chemotherapy (5.4 months), and increased OS (23.9 months) in comparison to treatment of physician's choice chemotherapy (17.5 months), in HR^+ HER2-low (defined as score of 1+ on immunohistochemistry or as score of 2+ on IHC and negative results on *in situ* hybridization) unresectable or metastatic BC patients who had been administered one/two previous lines of chemotherapy. Notably, BC patients with previous CDK4/6i treatment displayed a PFS benefit similar to those without previous CDK4/6i treatment (260). The safety profile of trastuzumab deruxtecan is consistent with that observed in previous $HER2^+$ BC patient-directed clinical trials (259), with no new toxicity-related concerns. Based on these data on HR^+ HER2-low BC, trastuzumab deruxtecan has also been FDA-approved for use in this cohort (260). The ADC sacituzumab govitecan acts toward transmembrane glycoprotein trophoblast cell-surface antigen 2 (Trop-2) and is connected to a topoisomerase I inhibitor SN-38 cytotoxic payload (261). Notoriously, Trop-2 is overexpressed in several epithelial cancers, and particularly, in more than 90% of $ER^+/HER2^-$ BC (262). In the TROPiCS-02 phase 3 clinical trial (NCT03901339), sacituzumab govitecan determined significantly longer PFS (5.5 months) compared to treatment of physician's choice chemotherapy (4 months), in three-median-line systemic therapy-pretreated $ER^+/HER2^-$ BC patients who were previously administered CDK4/6i. In addition, the PFS at 6 months and 12 months after sacituzumab govitecan or physician's choice chemotherapy were 46% *versus* 30%, and 21% *versus* 7%, respectively. Notably, clinical benefit in these BC patients were independent of Trop-2 expression status. Based upon these data, sacituzumab govitecan has been FDA-approved for use in this ER^+ cohort (263) (264) (265). Datopotamab deruxtecan is an ADC directed against Trop-2 using the same payload as trastuzumab deruxtecan. In the recent TROPION-PanTumor01 phase 1 clinical trial (NCT03401385), datopotamab deruxtecan monotherapy demonstrated promising anticancer activity (29% ORR, 85% DCR, and 41% CBR), and a manageable safety profile in heavily-pretreated $ER^+/HER2^-$ BC patients, the majority of whom had received previous CDK4/6i (266). The efficacy and safety profile of datopotamab deruxtecan, compared to investigator's choice of standard-of-care (SOC) chemotherapy, is being further assessed in the TROPION-Breast-01 phase 3 clinical study (NCT05104866) (267). Based upon these studies, and other recently completed/ongoing studies, ADCs will continue to emerge as an important and expanding option in our therapeutic arsenal. The optimal sequence of treatment, and the role that ADCs play in the setting of specific ET and CDK4/6i resistance scenarios, remains an active area of ongoing research.

7. Conclusions

Even though cyclin D and CDK4/6 have been studied for almost three decades, many facets of their biology and biochemistry have only been recognized recently and the complete functional spectrum of these crucial cell cycle-regulating kinases in BC cells remains elusive. The co-administration of small-molecule CDK4/6i and ET is the first-line treatment for advanced $ER^+/HER2^-$ BC patients. Despite the clear and significant clinical benefits of this combination, patients can still develop intrinsic or acquired drug resistance. Ongoing and future studies must be able to address these four main objectives: *1)* elucidating the full spectrum of CDK4/6i-mediated mechanisms of action; *2)* clarifying mechanisms underlying synergy between CDK4/6i and various endocrine therapies; *3)* improving current targeted treatment options post-progression; *4)* understanding mechanisms of resistance to dual CDK4/6i-ET treatment with suitable clinical validations:

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1) Despite our deep understanding of basic mechanisms related to cyclin-CDK activity, additional work is required to decipher more complex mechanisms of action outside of this canonical pathway. CDK4/6i directly suppress the enzymatic activity of CDK4/6; however, they may also act as indirect CDK2 inhibitors and impact other signal transduction pathways within the cancer cell. Clarifying these important factors may allow an improvement in the clinical activity of these agents, and a better understanding of potential resistance mechansisms. The hypothesis related to the importance of CDK4/6i-induced CDK2 inhibition is supported by the fact that CDK4/6 activity depends upon establishment of trimers comprising cyclin D and p21 or p27.

2) Clarification is still needed in relation to the mechanisms underlying synergy between CDK4/6i and various ETs in HR^+ BC. Particularly, a more in-depth investigation of the role of ET in augmenting and/or changing the therapy-mediated senescence phenotype, versus promoting apoptosis, is required. Insights like this will permit developing novel co-treatments with CDK4/6i plus ET and provide important information to improve our understanding of resistance in clinic trials. Further investigation is required to better understand the CDK4/6i-induced cellular senescence phenotype by clarifying *a)* to what extent CDK4/6i determine a SASP; *b)* how loss of p53 function can modify the senescence phenotype; and *c)* whether CDK4/CDK6i can also determine senescence in other proliferative cells (e.g., fibroblasts) in BC. Feasibility of single cell profiling, accurate genetic modeling *in vitro*/*in vivo*, and multi-omic profiling of CDK4/6i-treated cells will provide important insights related to these processes and new combinatorial treatments.

3) Though there are numerous treatment options post-CDK4/6i progression, there are still doubts regarding the optimum treatment-sequence and delineating a suitable biomarker strategy in this clinical setting is essential. ER^+ BC patients whose cancer remains dependent on ER signaling benefit more from switching ET backbone. Understanding the mechansims of CDK4/6i resistance on a personalized individual level may allow better therapeutic planning for each patient and delay the need for more toxic conventional chemotherapy. New targeted options exist or are in development that allow targeting of PI3K, AKT, and PARP, along with novel ADC therapies. However, the use of these targeted therapies is hampered by limited genomic testing and treatment-induced side effect profiles, which requires further investigation.

4) Finally, and most importantly, CDK4/6i resistance still represents a major challenge in clinical practice, and there is a wide array of potential resistance mechanisms. Indeed, clinical studies have been based on DNA sequencing of resistant tumors, and preclinical data have implicated disparate non-genomic resistance mechanisms such as stromal cell senescence, abnormal function of chromatin modifiers, and altered kinase signaling. Further investigation of CDK4/6i-ET treatment-resistant samples is needed, for instance through transcriptomic and/or epigenomic profiling at single cell resolution. A better understanding of these complex resistance pathways can have a tremendous potential to inform regarding new therapeutic approaches at a personalized level. Frequency estimation related to new mutations in CDK4/6i-resistant BC differ remarkably, and thus clarifying this topic is crucial as we work to elucidating all the pathways leading to drug resistance.

These four unanswered questions highlight some of the key areas in which our understanding of cyclin D-CDK4/6 biology and biochemistry will evolve in the coming years, with a therapeutic focus on development and testing of novel CDK4/6i in future BC clinical trials.

Abbreviations

ADCs: Antibody-drug conjugates
AI: Aromatase inhibitor/s
AP-1: Activator protein-1
AR: Androgen receptor
BC: Breast cancer
CAK: CDK-activating kinase
CBR: Clinical benefit rate
CDKs: Cyclin-dependent kinases
CDK1: Cyclin-dependent kinases 1
CDK2: Cyclin-dependent kinases 2
CDK2i: CDK2 inhibitor/s
CDK4: Cyclin-dependent kinases 4
CDK4/6i: CDK4/CDK6 inhibitor/s
CDK6: Cyclin-dependent kinases 6
CDK7: Cyclin-dependent kinases 7
CDK9: Cyclin-dependent kinases 9
CERAN: Complete ER antagonist
c-MET: Mesenchymal-epithelial transition factor
ctDNA: Circulating tumor DNA
DCR: Disease control rate
DNMT1: DNA methyltransferase 1
DOR: Duration of response
EGFR: Epidermal growth factor receptor
ER: Estrogen receptor
ER$^+$: ER-positive
ESR1: Estrogen receptor 1
ET: Endocrine therapy
FAK: Focal adhesion kinase
FDA: Food and Drug Administration
FGFR: Fibroblast growth factor receptor
FOXM1: Forkhead box M1
GAP: GTPase-activating protein
HER: Human epidermal growth factor receptor
HER2$^-$: HER2-negative
HER2$^+$: HER2-positive
HR$^+$: Hormone receptor-positive
IDFS: Invasive disease-free survival
INF: Interferon
MHC: Major histocompatibility complex
MTD: Maximum tolerated dose
mTORC1: mTOR complex 1
mTORC2: mTOR complex 2
NFAT: Nuclear factor of activated T-cells
NF1: Neurofibromin 1
ORR: Overall response rate
OS: Overall survival
PARP: Poly (ADP-ribose) polymerase
PD-L1: Programmed death-ligand 1

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PFS: Progression-free survival
PR: Partial response
PROTAC: Proteolysis targeting chimera
RP2D: Recommended phase 2 dose
RTK: Receptor tyrosine kinase
SARM: Selective androgen receptor modulator
SASP: Senescence-associated secretory phenotype
SD: Stable disease
SERCA: Selective ER covalent antagonists
SERD: Selective estrogen receptor degrader
SERM: Selective ER modulator
ShERPA: Selective human ER partial agonists
SIN1: Stress-activated protein kinase-interacting protein
Tregs: Regulatory T-cells
Trop-2: Trophoblast cell-surface antigen 2
TSC: Tuberous sclerosis complex
TSC1: Tuberous sclerosis complex 1
TSC2: Tuberous sclerosis complex 2

Declarations

Ethical approval

Not applicable

Consent for publication

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all the authors.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work in this paper.

Conflict of interest

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Author contributions

A.G. and A.P.K. conceptualized the manuscript topic; A.G., S.A.W., R.D.B., P.D., E.T., J.S., K.V., R.A.M.dB., U.S., A.B., and A.P.K. collated literature for the manuscript; A.G. and A.P.K. wrote the manuscript; A.G., S.A.W., R.D.B., K.CH.Y., H.Y.L., M.T., D.C., B.G., V.S., R.H.J., J.N., P.D., E.T., J.S., K.C., R.S.F., K.V., R.A.M.dB., U.S., A.B., and A.P.K. edited the manuscript; A.G. designed the figures. All authors have read and agreed to the submitted version of the manuscript.

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References

1. Matthews HK, Bertoli C, de Bruin RAM. Cell cycle control in cancer. Nat Rev Mol Cell Biol. 2022;23(1):74-88.
2. Grinshpun A, Tolaney SM, Burstein HJ, Jeselsohn R, Mayer EL. The dilemma of selecting a first line CDK4/6 inhibitor for hormone receptor-positive/HER2-negative metastatic breast cancer. NPJ Breast Cancer. 2023;9(1):15.
3. Stanciu IM, Parosanu AI, Orlov-Slavu C, Iaciu IC, Popa AM, Olaru CM, et al. Mechanisms of Resistance to CDK4/6 Inhibitors and Predictive Biomarkers of Response in HR+/HER2-Metastatic Breast Cancer-A Review of the Literature. Diagnostics (Basel). 2023;13(5).
4. Scirocchi F, Scagnoli S, Botticelli A, Di Filippo A, Napoletano C, Zizzari IG, et al. Immune effects of CDK4/6 inhibitors in patients with HR. EBioMedicine. 2022;79:104010.
5. Corti C, De Angelis C, Bianchini G, Malorni L, Giuliano M, Hamilton E, et al. Novel endocrine therapies: What is next in estrogen receptor positive, HER2 negative breast cancer? Cancer Treat Rev. 2023;117:102569.
6. Hartkopf AD, Grischke EM, Brucker SY. Endocrine-Resistant Breast Cancer: Mechanisms and Treatment. Breast Care (Basel). 2020;15(4):347-54.
7. Rasha F, Sharma M, Pruitt K. Mechanisms of endocrine therapy resistance in breast cancer. Mol Cell Endocrinol. 2021;532:111322.
8. Mittal A, Molto Valiente C, Tamimi F, Schlam I, Sammons S, Tolaney SM, et al. Filling the Gap after CDK4/6 Inhibitors: Novel Endocrine and Biologic Treatment Options for Metastatic Hormone Receptor Positive Breast Cancer. Cancers (Basel). 2023;15(7).
9. Zhou FH, Downton T, Freelander A, Hurwitz J, Caldon CE, Lim E. CDK4/6 inhibitor resistance in estrogen receptor positive breast cancer, a 2023 perspective. Front Cell Dev Biol. 2023;11:1148792.
10. Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. Lancet. 2020;395(10226):817-27.
11. Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2022;20(6):691-722.

12. Dickler MN, Tolaney SM, Rugo HS, Cortés J, Diéras V, Patt D, et al. MONARCH 1, A Phase II Study of Abemaciclib, a CDK4 and CDK6 Inhibitor, as a Single Agent, in Patients with Refractory HR. Clin Cancer Res. 2017;23(17):5218-24.
13. Hamilton E, Cortes J, Ozyilkan O, Chen SC, Petrakova K, Manikhas A, 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. 2022;195(1):55-64.
14. Chen P, Lee NV, Hu W, Xu M, Ferre RA, Lam H, et al. Spectrum and Degree of CDK Drug Interactions Predicts Clinical Performance. Mol Cancer Ther. 2016;15(10):2273-81.
15. Hafner M, Mills CE, Subramanian K, Chen C, Chung M, Boswell SA, et al. Multiomics Profiling Establishes the Polypharmacology of FDA-Approved CDK4/6 Inhibitors and the Potential for Differential Clinical Activity. Cell Chem Biol. 2019;26(8):1067-80.e8.
16. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther. 2004;3(11):1427-38.
17. Gelbert LM, Cai S, Lin X, Sanchez-Martinez C, Del Prado M, Lallena MJ, 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. 2014;32(5):825-37.
18. Klein ME, Kovatcheva M, Davis LE, Tap WD, Koff A. CDK4/6 Inhibitors: The Mechanism of Action May Not Be as Simple as Once Thought. Cancer Cell. 2018;34(1):9-20.
19. George MA, Qureshi S, Omene C, Toppmeyer DL, Ganesan S. Clinical and Pharmacologic Differences of CDK4/6 Inhibitors in Breast Cancer. Front Oncol. 2021;11:693104.
20. Torres-Guzmán R, Calsina B, Hermoso A, Baquero C, Alvarez B, Amat J, et al. Preclinical characterization of abemaciclib in hormone receptor positive breast cancer. Oncotarget. 2017;8(41):69493-507.
21. Cejuela M, Gil-Torralvo A, Castilla M, Domínguez-Cejudo M, Falcón A, Benavent M, et al. Abemaciclib, Palbociclib, and Ribociclib in Real-World Data: A Direct Comparison of First-Line Treatment for Endocrine-Receptor-Positive Metastatic Breast Cancer. Int J Mol Sci. 2023;24(10).
22. Becherini C, Visani L, Caini S, Bhattacharya IS, Kirby AM, Nader Marta G, 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. 2023;119:102586.
23. Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW, Jager A. Inhibiting CDK4/6 in Breast Cancer with Palbociclib, Ribociclib, and Abemaciclib: Similarities and Differences. Drugs. 2021;81(3):317-31.
24. Kwapisz D. Cyclin-dependent kinase 4/6 inhibitors in breast cancer: palbociclib, ribociclib, and abemaciclib. Breast Cancer Res Treat. 2017;166(1):41-54.
25. Bulat I, Maglakelidze M, Krastev B, Arkenau HT, Murias C, Baird RD, et al. 334P Lerociclib (G1T38), a continuously dosed oral CDK4/6 inhibitor, with fulvestrant in HR+/HER2- advanced breast cancer patients: Updated phase II results and dose selection. Annal Oncology. 2020;31(S380).
26. Xu B, Zhang Q, Zhang P, Hu X, Li W, Tong Z, et al. Dalpiciclib or placebo plus fulvestrant in hormone receptor-positive and HER2-negative advanced breast cancer: a randomized, phase 3 trial. Nat Med. 2021;27(11):1904-9.
27. Zhang P, Zhang Q, Tong Z, Sun T, Li W, Ouyang Q, et al. Dalpiciclib plus letrozole or anastrozole versus placebo plus letrozole or anastrozole as first-line treatment in patients with hormone receptor-positive, HER2-negative advanced breast cancer (DAWNA-2): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2023;24(6):646-57.
28. O'Leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, et al. The Genetic Landscape and Clonal Evolution of Breast Cancer Resistance to Palbociclib plus Fulvestrant in the PALOMA-3 Trial. Cancer Discov. 2018;8(11):1390-403.

29. Sawant M, Wilson A, Sridaran D, Mahajan K, O'Conor CJ, Hagemann IS, et al. Epigenetic reprogramming of cell cycle genes by ACK1 promotes breast cancer resistance to CDK4/6 inhibitor. Oncogene. 2023;42(29):2263-77.
30. Martínez-Alonso D, Malumbres M. Mammalian cell cycle cyclins. Semin Cell Dev Biol. 2020;107:28-35.
31. Knudsen ES, Kumarasamy V, Nambiar R, Pearson JD, Vail P, Rosenheck H, et al. CDK/cyclin dependencies define extreme cancer cell-cycle heterogeneity and collateral vulnerabilities. Cell Rep. 2022;38(9):110448.
32. Peissert S, Schlosser A, Kendel R, Kuper J, Kisker C. Structural basis for CDK7 activation by MAT1 and Cyclin H. Proc Natl Acad Sci U S A. 2020;117(43):26739-48.
33. Bertoli C, de Bruin RA. Turning cell cycle entry on its head. Elife. 2014;3:e03475.
34. Rubin SM, Sage J, Skotheim JM. Integrating Old and New Paradigms of G1/S Control. Mol Cell. 2020;80(2):183-92.
35. Hume S, Dianov GL, Ramadan K. A unified model for the G1/S cell cycle transition. Nucleic Acids Res. 2020;48(22):12483-501.
36. Engeland K. Cell cycle regulation: p53-p21-RB signaling. Cell Death Differ. 2022;29(5):946-60.
37. Pennycook BR, Vesela E, Peripolli S, Singh T, Barr AR, Bertoli C, et al. E2F-dependent transcription determines replication capacity and S phase length. Nat Commun. 2020;11(1):3503.
38. Fouad S, Hauton D, D'Angiolella V. E2F1: Cause and Consequence of DNA Replication Stress. Front Mol Biosci. 2020;7:599332.
39. Oki T, Nishimura K, Kitaura J, Togami K, Maehara A, Izawa K, et al. A novel cell-cycle-indicator, mVenus-p27K-, identifies quiescent cells and visualizes G0-G1 transition. Sci Rep. 2014;4:4012.
40. Narasimha AM, Kaulich M, Shapiro GS, Choi YJ, Sicinski P, Dowdy SF. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. Elife. 2014;3.
41. Kim S, Leong A, Kim M, Yang HW. CDK4/6 initiates Rb inactivation and CDK2 activity coordinates cell-cycle commitment and G1/S transition. Sci Rep. 2022;12(1):16810.
42. Sanidas I, Morris R, Fella KA, Rumde PH, Boukhali M, Tai EC, et al. A Code of Mono-phosphorylation Modulates the Function of RB. Mol Cell. 2019;73(5):985-1000.e6.
43. Caillot M, Bourgeais J, Dakik H, Costé É, Mazure NM, Lelièvre É, et al. Cyclin D1 targets hexokinase 2 to control aerobic glycolysis in myeloma cells. Oncogenesis. 2020;9(7):68.
44. Goodrich DW, Wang NP, Qian YW, Lee EY, Lee WH. The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle. Cell. 1991;67(2):293-302.
45. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell. 1999;98(6):859-69.
46. Topacio BR, Zatulovskiy E, Cristea S, Xie S, Tambo CS, Rubin SM, et al. Cyclin D-Cdk4,6 Drives Cell-Cycle Progression via the Retinoblastoma Protein's C-Terminal Helix. Mol Cell. 2019;74(4):758-70.e4.
47. Kaulich M, Link VM, Lapek JD, Lee YJ, Glass CK, Gonzalez DJ, et al. A Cdk4/6-dependent phosphorylation gradient regulates the early to late G1 phase transition. Sci Rep. 2021;11(1):14736.
48. Bertoli C, Skotheim JM, de Bruin RA. Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol. 2013;14(8):518-28.
49. Tetsu O, McCormick F. Proliferation of cancer cells despite CDK2 inhibition. Cancer Cell. 2003;3(3):233-45.
50. Malumbres M, Sotillo R, Santamaría D, Galán J, Cerezo A, Ortega S, et al. Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell. 2004;118(4):493-504.

51. Goel S, Bergholz JS, JJ. Z. Targeting CDK4 and CDK6 in cancer. Nature Reviews Cancer. 2022.
52. Lees E, Faha B, Dulic V, Reed SI, Harlow E. Cyclin E/cdk2 and cyclin A/cdk2 kinases associate with p107 and E2F in a temporally distinct manner. Genes Dev. 1992;6(10):1874-85.
53. Arias EE, Walter JC. Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. Genes Dev. 2007;21(5):497-518.
54. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nat Rev Cancer. 2017;17(2):93-115.
55. Ha SH, Ferrell JE. Thresholds and ultrasensitivity from negative cooperativity. Science. 2016;352(6288):990-3.
56. Blethrow JD, Glavy JS, Morgan DO, Shokat KM. Covalent capture of kinase-specific phosphopeptides reveals Cdk1-cyclin B substrates. Proc Natl Acad Sci U S A. 2008;105(5):1442-7.
57. Dephoure N, Zhou C, Villén J, Beausoleil SA, Bakalarski CE, Elledge SJ, et al. A quantitative atlas of mitotic phosphorylation. Proc Natl Acad Sci U S A. 2008;105(31):10762-7.
58. Gavet O, Pines J. Activation of cyclin B1-Cdk1 synchronizes events in the nucleus and the cytoplasm at mitosis. J Cell Biol. 2010;189(2):247-59.
59. Choi YJ, Anders L. Signaling through cyclin D-dependent kinases. Oncogene. 2014;33(15):1890-903.
60. Emond R, Griffiths JI, Grolmusz VK, Nath A, Chen J, Medina EF, et al. Cell facilitation promotes growth and survival under drug pressure in breast cancer. Nat Commun. 2023;14(1):3851.
61. Muise-Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tsichlis PN, Rosen N. Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem. 1998;273(45):29864-72.
62. Diehl JA, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev. 1998;12(22):3499-511.
63. Schmidt M, Fernandez de Mattos S, van der Horst A, Klompmaker R, Kops GJ, Lam EW, et al. Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. Mol Cell Biol. 2002;22(22):7842-52.
64. Peeper DS, Upton TM, Ladha MH, Neuman E, Zalvide J, Bernards R, et al. Ras signalling linked to the cell-cycle machinery by the retinoblastoma protein. Nature. 1997;386(6621):177-81.
65. Averous J, Fonseca BD, Proud CG. Regulation of cyclin D1 expression by mTORC1 signaling requires eukaryotic initiation factor 4E-binding protein 1. Oncogene. 2008;27(8):1106-13.
66. Harrod A, Lai CF, Goldsbrough I, Simmons GM, Oppermans N, Santos DB, et al. Genome engineering for estrogen receptor mutations reveals differential responses to anti-estrogens and new prognostic gene signatures for breast cancer. Oncogene. 2022;41(44):4905-15.
67. Xie D, Pei Q, Li J, Wan X, Ye T. Emerging Role of E2F Family in Cancer Stem Cells. Front Oncol. 2021;11:723137.
68. Giacinti C, Giordano A. RB and cell cycle progression. Oncogene. 2006;25(38):5220-7.
69. Rampioni Vinciguerra GL, Sonego M, Segatto I, Dall'Acqua A, Vecchione A, Baldassarre G, et al. CDK4/6 Inhibitors in Combination Therapies: Better in Company Than Alone: A Mini Review. Front Oncol. 2022;12:891580.
70. Jeffreys SA, Becker TM, Khan S, Soon P, Neubauer H, de Souza P, et al. Prognostic and Predictive Value of CCND1/Cyclin D1 Amplification in Breast Cancer With a Focus on Postmenopausal Patients: A Systematic Review and Meta-Analysis. Front Endocrinol (Lausanne). 2022;13:895729.
71. Cheng T, Wu Y, Liu Z, Yu Y, Sun S, Guo M, et al. CDKN2A-mediated molecular subtypes characterize the hallmarks of tumor microenvironment and guide precision medicine in triple-negative breast cancer. Front Immunol. 2022;13:970950.

72. Shagisultanova E, Crump LS, Borakove M, Hall JK, Rasti AR, Harrison BA, et al. Triple Targeting of Breast Tumors Driven by Hormonal Receptors and HER2. Mol Cancer Ther. 2022;21(1):48-57.
73. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. Genes Dev. 1997;11(7):847-62.
74. Bisi JE, Sorrentino JA, Roberts PJ, Tavares FX, Strum JC. Preclinical Characterization of G1T28: A Novel CDK4/6 Inhibitor for Reduction of Chemotherapy-Induced Myelosuppression. Mol Cancer Ther. 2016;15(5):783-93.
75. Guiley KZ, Stevenson JW, Lou K, Barkovich KJ, Kumarasamy V, Wijeratne TU, et al. p27 allosterically activates cyclin-dependent kinase 4 and antagonizes palbociclib inhibition. Science. 2019;366(6471).
76. Pennycook BR, Barr AR. Palbociclib-mediated cell cycle arrest can occur in the absence of the CDK inhibitors p21 and p27. Open Biol. 2021;11(11):210125.
77. Wu X, Yang X, Xiong Y, Li R, Ito T, Ahmed TA, et al. Distinct CDK6 complexes determine tumor cell response to CDK4/6 inhibitors and degraders. Nat Cancer. 2021;2(4):429-43.
78. Wander SA, O'Brien N, Litchfield LM, O'Dea D, Morato Guimaraes C, Slamon DJ, et al. Targeting CDK4 and 6 in Cancer Therapy: Emerging Preclinical Insights Related to Abemaciclib. Oncologist. 2022;27(10):811-21.
79. Litchfield LM, Boehnke K, Brahmachary M, Mur C, Bi C, Stephens JR, et al. Combined inhibition of PIM and CDK4/6 suppresses both mTOR signaling and Rb phosphorylation and potentiates PI3K inhibition in cancer cells. Oncotarget. 2020;11(17):1478-92.
80. Watt AC, Goel S. Cellular mechanisms underlying response and resistance to CDK4/6 inhibitors in the treatment of hormone receptor-positive breast cancer. Breast Cancer Res. 2022;24(1):17.
81. Loibl S, Furlanetto J. Integrating CDK4/6 inhibitors in the treatment of patients with early breast cancer. Breast. 2022;62 Suppl 1(Suppl 1):S70-S9.
82. Pack LR, Daigh LH, Chung M, Meyer T. Clinical CDK4/6 inhibitors induce selective and immediate dissociation of p21 from cyclin D-CDK4 to inhibit CDK2. Nat Commun. 2021;12(1):3356.
83. Li Q, Jiang B, Guo J, Shao H, Del Priore IS, Chang Q, et al. INK4 Tumor Suppressor Proteins Mediate Resistance to CDK4/6 Kinase Inhibitors. Cancer Discov. 2022;12(2):356-71.
84. Wagner V, Gil J. Senescence as a therapeutically relevant response to CDK4/6 inhibitors. Oncogene. 2020;39(29):5165-76.
85. Wang B, Varela-Eirin M, Brandenburg SM, Hernandez-Segura A, van Vliet T, Jongbloed EM, et al. Pharmacological CDK4/6 inhibition reveals a p53-dependent senescent state with restricted toxicity. EMBO J. 2022;41(6):e108946.
86. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, et al. Cellular Senescence: Defining a Path Forward. Cell. 2019;179(4):813-27.
87. Watt AC, Cejas P, DeCristo MJ, Metzger-Filho O, Lam EYN, Qiu X, et al. CDK4/6 inhibition reprograms the breast cancer enhancer landscape by stimulating AP-1 transcriptional activity. Nat Cancer. 2021;2(1):34-48.
88. Martínez-Zamudio RI, Roux PF, de Freitas JANL, Robinson L, Doré G, Sun B, et al. AP-1 imprints a reversible transcriptional programme of senescent cells. Nat Cell Biol. 2020;22(7):842-55.
89. Walter DM, Yates TJ, Ruiz-Torres M, Kim-Kiselak C, Gudiel AA, Deshpande C, et al. RB constrains lineage fidelity and multiple stages of tumour progression and metastasis. Nature. 2019;569(7756):423-7.
90. Vijayaraghavan S, Karakas C, Doostan I, Chen X, Bui T, Yi M, et al. CDK4/6 and autophagy inhibitors synergistically induce senescence in Rb positive cytoplasmic cyclin E negative cancers. Nat Commun. 2017;8:15916.

91. Klapp V, Buqué A, Bloy N, Sato A, Yamazaki T, Zhou XK, et al. Cellular senescence in the response of HR. J Transl Med. 2023;21(1):110.
92. Kumari R, Jat P. Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype. Front Cell Dev Biol. 2021;9:645593.
93. Schmitt CA, Wang B, Demaria M. Senescence and cancer - role and therapeutic opportunities. Nat Rev Clin Oncol. 2022;19(10):619-36.
94. Maskey RS, Wang F, Lehman E, Wang Y, Emmanuel N, Zhong W, et al. Sustained mTORC1 activity during palbociclib-induced growth arrest triggers senescence in ER+ breast cancer cells. Cell Cycle. 2021;20(1):65-80.
95. Manohar S, Estrada ME, Uliana F, Vuina K, Alvarez PM, de Bruin RAM, et al. Genome homeostasis defects drive enlarged cells into senescence. Mol Cell. 2023;83(22):4032-46.e6.
96. Crozier L, Foy R, Adib R, Kar A, Holt JA, Pareri AU, et al. CDK4/6 inhibitor-mediated cell overgrowth triggers osmotic and replication stress to promote senescence. Mol Cell. 2023;83(22):4062-77.e5.
97. Foy R, Crozier L, Pareri AU, Valverde JM, Park BH, Ly T, et al. Oncogenic signals prime cancer cells for toxic cell overgrowth during a G1 cell cycle arrest. Mol Cell. 2023;83(22):4047-61.e6.
98. Romero-Pozuelo J, Figlia G, Kaya O, Martin-Villalba A, Teleman AA. Cdk4 and Cdk6 Couple the Cell-Cycle Machinery to Cell Growth via mTORC1. Cell Rep. 2020;31(2):107504.
99. Gong X, Litchfield LM, Webster Y, Chio LC, Wong SS, Stewart TR, et al. Genomic Aberrations that Activate D-type Cyclins Are Associated with Enhanced Sensitivity to the CDK4 and CDK6 Inhibitor Abemaciclib. Cancer Cell. 2017;32(6):761-76.e6.
100. Zou X, Ray D, Aziyu A, Christov K, Boiko AD, Gudkov AV, et al. Cdk4 disruption renders primary mouse cells resistant to oncogenic transformation, leading to Arf/p53-independent senescence. Genes Dev. 2002;16(22):2923-34.
101. Michaud K, Solomon DA, Oermann E, Kim JS, Zhong WZ, Prados MD, et al. Pharmacologic inhibition of cyclin-dependent kinases 4 and 6 arrests the growth of glioblastoma multiforme intracranial xenografts. Cancer Res. 2010;70(8):3228-38.
102. Yoshida A, Lee EK, Diehl JA. Induction of Therapeutic Senescence in Vemurafenib-Resistant Melanoma by Extended Inhibition of CDK4/6. Cancer Res. 2016;76(10):2990-3002.
103. Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, et al. CDK4/6 inhibition triggers anti-tumour immunity. Nature. 2017;548(7668):471-5.
104. Johnston S, Puhalla S, Wheatley D, Ring A, Barry P, Holcombe C, et al. Randomized Phase II Study Evaluating Palbociclib in Addition to Letrozole as Neoadjuvant Therapy in Estrogen Receptor-Positive Early Breast Cancer: PALLET Trial. J Clin Oncol. 2019;37(3):178-89.
105. Kervadec J, Priault M. Advances in Bcl-xL Research 2.0. Int J Mol Sci. 2023;24(11).
106. Valentini E, Di Martile M, Brignone M, Di Caprio M, Manni I, Chiappa M, et al. Bcl-2 family inhibitors sensitize human cancer models to therapy. Cell Death Dis. 2023;14(7):441.
107. Alcon C, Gómez Tejeda Zañudo J, Albert R, Wagle N, Scaltriti M, Letai A, et al. ER+ Breast Cancer Strongly Depends on MCL-1 and BCL-xL Anti-Apoptotic Proteins. Cells. 2021;10(7).
108. Roberts AW, Huang D. Targeting BCL2 With BH3 Mimetics: Basic Science and Clinical Application of Venetoclax in Chronic Lymphocytic Leukemia and Related B Cell Malignancies. Clin Pharmacol Ther. 2017;101(1):89-98.
109. Muttiah C, Whittle JR, Oakman C, Lindeman GJ. PALVEN: phase Ib trial of palbociclib, letrozole and venetoclax in estrogen receptor- and BCL2-positive advanced breast cancer. Future Oncol. 2022;18(15):1805-16.
110. Lindeman GJ, Fernando TM, Bowen R, Jerzak KJ, Song X, Decker T, 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. 2022;28(15):3256-67.
111. Qi J, Ouyang Z. Targeting CDK4/6 for Anticancer Therapy. Biomedicines. 2022;10(3).
112. Choi YJ, Li X, Hydbring P, Sanda T, Stefano J, Christie AL, et al. The requirement for cyclin D function in tumor maintenance. Cancer Cell. 2012;22(4):438-51.
113. Xiao M, Benoit A, Hasmim M, Duhem C, Vogin G, Berchem G, et al. Targeting Cytoprotective Autophagy to Enhance Anticancer Therapies. Front Oncol. 2021;11:626309.
114. Bestion E, Raymond E, Mezouar S, Halfon P. Update on Autophagy Inhibitors in Cancer: Opening up to a Therapeutic Combination with Immune Checkpoint Inhibitors. Cells. 2023;12(13).
115. Kim HM, Koo JS. The Role of Autophagy in Breast Cancer Metastasis. Biomedicines. 2023;11(2).
116. Pawlowska E, Szczepanska J, Szatkowska M, Blasiak J. An Interplay between Senescence, Apoptosis and Autophagy in Glioblastoma Multiforme-Role in Pathogenesis and Therapeutic Perspective. Int J Mol Sci. 2018;19(3).
117. Cassidy LD, Narita M. Autophagy at the intersection of aging, senescence, and cancer. Mol Oncol. 2022;16(18):3259-75.
118. Wu Q, Sharma D. Autophagy and Breast Cancer: Connected in Growth, Progression, and Therapy. Cells. 2023;12(8).
119. Brown NE, Jeselsohn R, Bihani T, Hu MG, Foltopoulou P, Kuperwasser C, et al. Cyclin D1 activity regulates autophagy and senescence in the mammary epithelium. Cancer Res. 2012;72(24):6477-89.
120. Song D, Lian Y, Zhang L. The potential of activator protein 1 (AP-1) in cancer targeted therapy. Front Immunol. 2023;14:1224892.
121. Wu Z, Nicoll M, Ingham RJ. AP-1 family transcription factors: a diverse family of proteins that regulate varied cellular activities in classical hodgkin lymphoma and ALK+ ALCL. Exp Hematol Oncol. 2021;10(1):4.
122. Sinha KK, Bilokapic S, Du Y, Malik D, Halic M. Histone modifications regulate pioneer transcription factor cooperativity. Nature. 2023;619(7969):378-84.
123. Sobhani N, Fassl A, Mondani G, Generali D, Otto T. Targeting Aberrant FGFR Signaling to Overcome CDK4/6 Inhibitor Resistance in Breast Cancer. Cells. 2021;10(2).
124. Clark AS, Makhlin I, DeMichele A. Setting the Pick: Can PI3K Inhibitors Circumvent CDK4/6 Inhibitor Resistance? Clin Cancer Res. 2021;27(2):371-3.
125. Zhao M, Scott S, Evans KW, Yuca E, Saridogan T, Zheng X, et al. Combining Neratinib with CDK4/6, mTOR, and MEK Inhibitors in Models of HER2-positive Cancer. Clin Cancer Res. 2021;27(6):1681-94.
126. Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, et al. Early Adaptation and Acquired Resistance to CDK4/6 Inhibition in Estrogen Receptor-Positive Breast Cancer. Cancer Res. 2016;76(8):2301-13.
127. Klein EA, Assoian RK. Transcriptional regulation of the cyclin D1 gene at a glance. J Cell Sci. 2008;121(Pt 23):3853-7.
128. Li Z, Zou W, Zhang J, Zhang Y, Xu Q, Li S, et al. Mechanisms of CDK4/6 Inhibitor Resistance in Luminal Breast Cancer. Front Pharmacol. 2020;11:580251.
129. Goel S, Wang Q, Watt AC, Tolaney SM, Dillon DA, Li W, et al. Overcoming Therapeutic Resistance in HER2-Positive Breast Cancers with CDK4/6 Inhibitors. Cancer Cell. 2016;29(3):255-69.
130. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell. 2011;19(1):58-71.
131. He Z, Houghton PJ, Williams TM, Shen C. Regulation of DNA duplication by the mTOR signaling pathway. Cell Cycle. 2021;20(8):742-51.

132. Tolaney SM, Wardley AM, Zambelli S, Hilton JF, Troso-Sandoval TA, Ricci F, et al. Abemaciclib plus trastuzumab with or without fulvestrant versus trastuzumab plus standard-of-care chemotherapy in women with hormone receptor-positive, HER2-positive advanced breast cancer (monarcHER): a randomised, open-label, phase 2 trial. Lancet Oncol. 2020;21(6):763-75.
133. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer. 2023;22(1):138.
134. Pascual J, Lim JSJ, Macpherson IR, Armstrong AC, Ring A, Okines AFC, 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. 2021;11(1):92-107.
135. Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, et al. CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation. Cancer Discov. 2018;8(2):216-33.
136. Jerby-Arnon L, Shah P, Cuoco MS, Rodman C, Su MJ, Melms JC, et al. A Cancer Cell Program Promotes T Cell Exclusion and Resistance to Checkpoint Blockade. Cell. 2018;175(4):984-97.e24.
137. Lelliott EJ, Kong IY, Zethoven M, Ramsbottom KM, Martelotto LG, Meyran D, et al. CDK4/6 Inhibition Promotes Antitumor Immunity through the Induction of T-cell Memory. Cancer Discov. 2021;11(10):2582-601.
138. Haggerty C, Kretzmer H, Riemenschneider C, Kumar AS, Mattei AL, Bailly N, et al. Dnmt1 has de novo activity targeted to transposable elements. Nat Struct Mol Biol. 2021;28(7):594-603.
139. Uzhachenko RV, Bharti V, Ouyang Z, Blevins A, Mont S, Saleh N, et al. Metabolic modulation by CDK4/6 inhibitor promotes chemokine-mediated recruitment of T cells into mammary tumors. Cell Rep. 2021;35(12):109271.
140. Lai AY, Sorrentino JA, Dragnev KH, Weiss JM, Owonikoko TK, Rytlewski JA, et al. CDK4/6 inhibition enhances antitumor efficacy of chemotherapy and immune checkpoint inhibitor combinations in preclinical models and enhances T-cell activation in patients with SCLC receiving chemotherapy. J Immunother Cancer. 2020;8(2).
141. Whittle JR, Vaillant F, Surgenor E, Policheni AN, Giner G, Capaldo BD, et al. Dual Targeting of CDK4/6 and BCL2 Pathways Augments Tumor Response in Estrogen Receptor-Positive Breast Cancer. Clin Cancer Res. 2020;26(15):4120-34.
142. Schaer DA, Beckmann RP, Dempsey JA, Huber L, Forest A, Amaladas N, 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. 2018;22(11):2978-94.
143. Heckler M, Ali LR, Clancy-Thompson E, Qiang L, Ventre KS, Lenehan P, et al. Inhibition of CDK4/6 Promotes CD8 T-cell Memory Formation. Cancer Discov. 2021;11(10):2564-81.
144. Peuker CA, Yaghobramzi S, Grunert C, Keilholz L, Gjerga E, Hennig S, et al. Treatment with ribociclib shows favourable immunomodulatory effects in patients with hormone receptor-positive breast cancer-findings from the RIBECCA trial. Eur J Cancer. 2022;162:45-55.
145. Hurvitz SA, Martin M, Press MF, Chan D, Fernandez-Abad M, Petru E, et al. Potent Cell-Cycle Inhibition and Upregulation of Immune Response with Abemaciclib and Anastrozole in neoMONARCH, Phase II Neoadjuvant Study in HR. Clin Cancer Res. 2020;26(3):566-80.
146. De Angelis C, Fu X, Cataldo ML, Nardone A, Pereira R, Veeraraghavan J, 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. 2021;27(17):4870-82.
147. Walker AJ, Wedam S, Amiri-Kordestani L, Bloomquist E, Tang S, Sridhara R, et al. FDA Approval of Palbociclib in Combination with Fulvestrant for the Treatment of Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer. Clin Cancer Res. 2016;22(20):4968-72.
148. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 2016;375(20):1925-36.
149. Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, 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. 2016;17(4):425-39.
150. Shah A, Bloomquist E, Tang S, Fu W, Bi Y, Liu Q, et al. FDA Approval: Ribociclib for the Treatment of Postmenopausal Women with Hormone Receptor-Positive, HER2-Negative Advanced or Metastatic Breast Cancer. Clin Cancer Res. 2018;24(13):2999-3004.
151. Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im SA, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol. 2018;36(24):2465-72.
152. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, 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. 2019;30(11):1842.
153. Sledge GW, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. J Clin Oncol. 2017;35(25):2875-84.
154. Johnston S, Martin M, Di Leo A, Im SA, Awada A, Forrester T, et al. MONARCH 3 final PFS: a randomized study of abemaciclib as initial therapy for advanced breast cancer. NPJ Breast Cancer. 2019;5:5.
155. Takahashi M, Tokunaga E, Mori J, Tanizawa Y, van der Walt JS, Kawaguchi T, et al. 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. 2022;29(1):174-84.
156. Raheem F, Ofori H, Simpson L, Shah V. Abemaciclib: The First FDA-Approved CDK4/6 Inhibitor for the Adjuvant Treatment of HR+ HER2- Early Breast Cancer. Ann Pharmacother. 2022:10600280211073322.
157. Cristofanilli M, Rugo HS, Im SA, Slamon DJ, Harbeck N, Bondarenko I, et al. Overall Survival with Palbociclib and Fulvestrant in Women with HR+/HER2- ABC: Updated Exploratory Analyses of PALOMA-3, a Double-blind, Phase III Randomized Study. Clin Cancer Res. 2022;28(16):3433-42.
158. Slamon DJ, Neven P, Chia S, Jerusalem G, De Laurentiis M, Im S, et al. Ribociclib plus fulvestrant for postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer in the phase III randomized MONALEESA-3 trial: updated overall survival. Ann Oncol. 2021;32(8):1015-24.
159. Sledge GW, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. The Effect of Abemaciclib Plus Fulvestrant on Overall Survival in Hormone Receptor-Positive, ERBB2-Negative Breast Cancer That Progressed on Endocrine Therapy-MONARCH 2: A Randomized Clinical Trial. JAMA Oncol. 2020;6(1):116-24.
160. Lu YS, Im SA, Colleoni M, Franke F, Bardia A, Cardoso F, et al. Updated Overall Survival of Ribociclib plus Endocrine Therapy versus Endocrine Therapy Alone in Pre- and Perimenopausal Patients with HR+/HER2- Advanced Breast Cancer in MONALEESA-7: A Phase III Randomized Clinical Trial. Clin Cancer Res. 2022;28(5):851-9.
161. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Hart L, et al. Overall Survival with Ribociclib plus Letrozole in Advanced Breast Cancer. N Engl J Med. 2022;386(10):942-50.
162. Finn RS, Rugo HS, Dieras VC, Harbeck N, Im S-A, Gelmon KA, et al. Overall survival (OS) with first-line palbociclib plus letrozole (PAL+LET) versus placebo plus letrozole (PBO+LET) in women with estrogen receptor–positive/human epidermal growth factor receptor 2–

negative advanced breast cancer (ER+/HER2− ABC): Analyses from PALOMA-2. (Abstract). Journal of Clinical Oncology. 2022;40(17).
163. Goetz MP, Toi M, Huober J, Sohn J, Tredan O, Park IH, et al. LBA15 MONARCH 3: Interim overall survival (OS) results of abemaciclib plus a nonsteroidal aromatase inhibitor (NSAI) in patients (pts) with HR+, HER2-advanced breast cancer (ABC). (Abstract). Ann Oncol. 2022b;33.
164. Mayer EL, Dueck AC, Martin M, Rubovszky G, Burstein HJ, Bellet-Ezquerra M, et al. Palbociclib with adjuvant endocrine therapy in early breast cancer (PALLAS): interim analysis of a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2021;22(2):212-22.
165. Loibl S, Marmé F, Martin M, Untch M, Bonnefoi H, Kim SB, et al. Palbociclib for Residual High-Risk Invasive HR-Positive and HER2-Negative Early Breast Cancer-The Penelope-B Trial. J Clin Oncol. 2021;39(14):1518-30.
166. Johnston SRD, Toi M, O'Shaughnessy J, Rastogi P, Campone M, Neven P, et al. Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncol. 2023;24(1):77-90.
167. Slamon DJ, Fasching PA, Hurvitz S, Chia S, Crown J, Martín M, et al. Rationale and trial design of NATALEE: a Phase III trial of adjuvant ribociclib + endocrine therapy. Ther Adv Med Oncol. 2023;15:17588359231178125.
168. Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol. 2017;35(32):3638-46.
169. Goetz MP, Martin M, Tokunaga E, Park IH, Huober J, Toi M, et al. Health-Related Quality of Life in MONARCH 3: Abemaciclib plus an Aromatase Inhibitor as Initial Therapy in HR+, HER2- Advanced Breast Cancer. Oncologist. 2020;25(9):e1346-e54.
170. Scheidemann ER, Shajahan-Haq AN. Resistance to CDK4/6 Inhibitors in Estrogen Receptor-Positive Breast Cancer. Int J Mol Sci. 2021;22(22).
171. Kong T, Xue Y, Cencic R, Zhu X, Monast A, Fu Z, et al. eIF4A Inhibitors Suppress Cell-Cycle Feedback Response and Acquired Resistance to CDK4/6 Inhibition in Cancer. Mol Cancer Ther. 2019;18(11):2158-70.
172. Griffiths JI, Chen J, Cosgrove PA, O'Dea A, Sharma P, Ma C, et al. Serial single-cell genomics reveals convergent subclonal evolution of resistance as early-stage breast cancer patients progress on endocrine plus CDK4/6 therapy. Nat Cancer. 2021;2(6):658-71.
173. Wander SA, Cohen O, Gong X, Johnson GN, Buendia-Buendia JE, Lloyd MR, 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. 2020;10(8):1174-93.
174. Asghar US, Kanani R, Roylance R, Mittnacht S. Systematic Review of Molecular Biomarkers Predictive of Resistance to CDK4/6 Inhibition in Metastatic Breast Cancer. JCO Precis Oncol. 2022;6:e2100002.
175. Goetz MP, Hamilton EP, Campone M, Hurvitz SA, Cortes J, Johnston SRD, et al. Acquired genomic alterations in circulating tumor DNA from patients receiving abemaciclib alone or in combination with endocrine therapy. (Abstract). Journal of Clinical Oncology. 2020;38(15).
176. Fassl A, Geng Y, Sicinski P. CDK4 and CDK6 kinases: From basic science to cancer therapy. Science. 2022;375(6577):eabc1495.
177. Condorelli R, Spring L, O'Shaughnessy J, Lacroix L, Bailleux C, Scott V, et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol. 2018;29(3):640-5.
178. Kumarasamy V, Nambiar R, Wang J, Rosenheck H, Witkiewicz AK, Knudsen ES. RB loss determines selective resistance and novel vulnerabilities in ER-positive breast cancer models. Oncogene. 2022;41(27):3524-38.

179. Palafox M, Monserrat L, Bellet M, Villacampa G, Gonzalez-Perez A, Oliveira M, et al. High p16 expression and heterozygous RB1 loss are biomarkers for CDK4/6 inhibitor resistance in ER. Nat Commun. 2022;13(1):5258.
180. Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, et al. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene. 2017;36(16):2255-64.
181. Al-Qasem AJ, Alves CL, Ehmsen S, Tuttolomondo M, Terp MG, Johansen LE, et al. Co-targeting CDK2 and CDK4/6 overcomes resistance to aromatase and CDK4/6 inhibitors in ER+ breast cancer. NPJ Precis Oncol. 2022;6(1):68.
182. Chen ZG, Saba NF, Teng Y. The diverse functions of FAT1 in cancer progression: good, bad, or ugly? J Exp Clin Cancer Res. 2022;41(1):248.
183. Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, et al. Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. Cancer Cell. 2018;34(6):893-905.e8.
184. Martin D, Degese MS, Vitale-Cross L, Iglesias-Bartolome R, Valera JLC, Wang Z, et al. Assembly and activation of the Hippo signalome by FAT1 tumor suppressor. Nat Commun. 2018;9(1):2372.
185. Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, et al. Expression of Yes-associated protein in common solid tumors. Hum Pathol. 2008;39(11):1582-9.
186. Cornell L, Wander SA, Visal T, Wagle N, Shapiro GI. MicroRNA-Mediated Suppression of the TGF-β Pathway Confers Transmissible and Reversible CDK4/6 Inhibitor Resistance. Cell Rep. 2019;26(10):2667-80.e7.
187. Goel S, DeCristo MJ, McAllister SS, Zhao JJ. CDK4/6 Inhibition in Cancer: Beyond Cell Cycle Arrest. Trends Cell Biol. 2018;28(11):911-25.
188. Turner NC, Liu Y, Zhu Z, Loi S, Colleoni M, Loibl S, et al. Cyclin E1 Expression and Palbociclib Efficacy in Previously Treated Hormone Receptor-Positive Metastatic Breast Cancer. J Clin Oncol. 2019;37(14):1169-78.
189. Pandey K, Park N, Park KS, Hur J, Cho YB, Kang M, et al. Combined CDK2 and CDK4/6 Inhibition Overcomes Palbociclib Resistance in Breast Cancer by Enhancing Senescence. Cancers (Basel). 2020;12(12).
190. Su P, Zhang M, Kang X. Targeting c-Met in the treatment of urologic neoplasms: Current status and challenges. Front Oncol. 2023;13:1071030.
191. Li X, Combs JD, Salaita K, Shu X. Polarized focal adhesion kinase activity within a focal adhesion during cell migration. Nat Chem Biol. 2023.
192. Zhang C, Stockwell SR, Elbanna M, Ketteler R, Freeman J, Al-Lazikani B, et al. Signalling involving MET and FAK supports cell division independent of the activity of the cell cycle-regulating CDK4/6 kinases. Oncogene. 2019;38(30):5905-20.
193. Karadkhelkar NM, Lin M, Eubanks LM, Janda KD. Demystifying the Druggability of the MYC Family of Oncogenes. J Am Chem Soc. 2023;145(6):3259-69.
194. Gao FY, Li XT, Xu K, Wang RT, Guan XX. c-MYC mediates the crosstalk between breast cancer cells and tumor microenvironment. Cell Commun Signal. 2023;21(1):28.
195. Fallah Y, Brundage J, Allegakoen P, Shajahan-Haq AN. MYC-Driven Pathways in Breast Cancer Subtypes. Biomolecules. 2017;7(3).
196. Freeman-Cook K, Hoffman RL, Miller N, Almaden J, Chionis J, Zhang Q, et al. Expanding control of the tumor cell cycle with a CDK2/4/6 inhibitor. Cancer Cell. 2021;39(10):1404-21.e11.
197. Mo H, Liu X, Xue Y, Chen H, Guo S, Li Z, et al. S6K1 amplification confers innate resistance to CDK4/6 inhibitors through activating c-Myc pathway in patients with estrogen receptor-positive breast cancer. Mol Cancer. 2022;21(1):171.

198. Alves CL, Ehmsen S, Terp MG, Portman N, Tuttolomondo M, Gammelgaard OL, et al. Co-targeting CDK4/6 and AKT with endocrine therapy prevents progression in CDK4/6 inhibitor and endocrine therapy-resistant breast cancer. Nat Commun. 2021;12(1):5112.
199. O'Brien NA, McDermott MSJ, Conklin D, Luo T, Ayala R, Salgar S, et al. Targeting activated PI3K/mTOR signaling overcomes acquired resistance to CDK4/6-based therapies in preclinical models of hormone receptor-positive breast cancer. Breast Cancer Res. 2020;22(1):89.
200. de Leeuw R, McNair C, Schiewer MJ, Neupane NP, Brand LJ, Augello MA, et al. MAPK Reliance via Acquired CDK4/6 Inhibitor Resistance in Cancer. Clin Cancer Res. 2018;24(17):4201-14.
201. Gomes I, Abreu C, Costa L, Casimiro S. The Evolving Pathways of the Efficacy of and Resistance to CDK4/6 Inhibitors in Breast Cancer. Cancers (Basel). 2023;15(19).
202. Hanker AB, Sudhan DR, Arteaga CL. Overcoming Endocrine Resistance in Breast Cancer. Cancer Cell. 2020;37(4):496-513.
203. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. Cell. 2017;169(3):381-405.
204. Zhang J, Xu K, Liu P, Geng Y, Wang B, Gan W, et al. Inhibition of Rb Phosphorylation Leads to mTORC2-Mediated Activation of Akt. Mol Cell. 2016;62(6):929-42.
205. Olmez I, Zhang Y, Manigat L, Benamar M, Brenneman B, Nakano I, et al. Combined c-Met/Trk Inhibition Overcomes Resistance to CDK4/6 Inhibitors in Glioblastoma. Cancer Res. 2018;78(15):4360-9.
206. Knudsen ES, Kumarasamy V, Ruiz A, Sivinski J, Chung S, Grant A, et al. Cell cycle plasticity driven by MTOR signaling: integral resistance to CDK4/6 inhibition in patient-derived models of pancreatic cancer. Oncogene. 2019;38(18):3355-70.
207. Kumarasamy V, Vail P, Nambiar R, Witkiewicz AK, Knudsen ES. Functional Determinants of Cell Cycle Plasticity and Sensitivity to CDK4/6 Inhibition. Cancer Res. 2021;81(5):1347-60.
208. Fingar DC, Richardson CJ, Tee AR, Cheatham L, Tsou C, Blenis J. mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor 4E. Mol Cell Biol. 2004;24(1):200-16.
209. Michaloglou C, Crafter C, Siersbaek R, Delpuech O, Curwen JO, Carnevalli LS, 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. 2018;17(5):908-20.
210. Wander SA, Keenan JC, Niemierko A, Juric D, Spring LM, Supko J, et al. Abstract PD13-07: Combination therapy with the AKT inhibitor, ipatasertib, endocrine therapy, and a CDK4/6 inhibitor for hormone receptor positive (HR+)/HER2 negative metastatic breast cancer (MBC): results from the phase I TAKTIC trial. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2022.
211. Vora SR, Juric D, Kim N, Mino-Kenudson M, Huynh T, Costa C, et al. CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell. 2014;26(1):136-49.
212. Jansen VM, Bhola NE, Bauer JA, Formisano L, Lee KM, Hutchinson KE, et al. Kinome-Wide RNA Interference Screen Reveals a Role for PDK1 in Acquired Resistance to CDK4/6 Inhibition in ER-Positive Breast Cancer. Cancer Res. 2017;77(9):2488-99.
213. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. Trends Biochem Sci. 2005;30(11):630-41.
214. Santamaría D, Barrière C, Cerqueira A, Hunt S, Tardy C, Newton K, et al. Cdk1 is sufficient to drive the mammalian cell cycle. Nature. 2007;448(7155):811-5.
215. Freeman-Cook KD, Hoffman RL, Behenna DC, Boras B, Carelli J, Diehl W, et al. Discovery of PF-06873600, a CDK2/4/6 Inhibitor for the Treatment of Cancer. J Med Chem. 2021;64(13):9056-77.

216. Arora M, Moser J, Hoffman TE, Watts LP, Min M, Musteanu M, et al. Rapid adaptation to CDK2 inhibition exposes intrinsic cell-cycle plasticity. Cell. 2023;186(12):2628-43.e21.
217. Larochelle S, Pandur J, Fisher RP, Salz HK, Suter B. Cdk7 is essential for mitosis and for in vivo Cdk-activating kinase activity. Genes Dev. 1998;12(3):370-81.
218. Glover-Cutter K, Larochelle S, Erickson B, Zhang C, Shokat K, Fisher RP, et al. TFIIH-associated Cdk7 kinase functions in phosphorylation of C-terminal domain Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II. Mol Cell Biol. 2009;29(20):5455-64.
219. Coombes RC, Howell S, Lord SR, Kenny L, Mansi J, Mitri Z, 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. 2023;14(1):4444.
220. Wilson G, Sava G, Vuina K, Huard C, Meneguello L, Coulombe-Huntington J, et al. Active Growth Signalling Promotes Senescence and Cancer Cell Sensitivity to CDK7 Inhibition. Molecular Cell. 2023;Publication Status: Accepted.
221. Mayer EL, Ren Y, Wagle N, Mahtani R, Ma C, DeMichele A, et al. Abstract 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. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
222. Yardley DA, Abu-Khalaf M, Boni V, Brufsky A, Emens LA, Gutierrez M, et al. Abstract OT2-06-04: MORPHEUS: A phase Ib/II trial platform evaluating the safety and efficacy of multiple cancer immunotherapy combinations in patients with hormone receptor–positive and triple-negative breast cancer. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Res; 2019.
223. Kalinsky K, Accordino MK, Chiuzan C, Mundi PS, Sakach E, Sathe C, et al. Randomized Phase II Trial of Endocrine Therapy With or Without Ribociclib After Progression on Cyclin-Dependent Kinase 4/6 Inhibition in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer: MAINTAIN Trial. J Clin Oncol. 2023:JCO2202392.
224. Albanell J, Pérez-García JM, Gil-Gil M, Curigliano G, Ruíz-Borrego M, Comerma L, et al. Palbociclib Rechallenge for Hormone Receptor-Positive/HER-Negative Advanced Breast Cancer: Findings from the Phase II BioPER Trial. Clin Cancer Res. 2023;29(1):67-80.
225. Patel MR, Juric D, Henick BS, Duska LR, Wu R, Guo J, et al. BLU-222, an oral, potent, and selective CDK2 inhibitor, in patients with advanced solid tumors: Phase 1 monotherapy dose escalation. (Abstract). Journal of Clinical Oncology. 2023;41(16):3095.
226. Yap TA, Elhaddad AM, Grisham RN, Hamm JT, Marks DK, Shapiro G, et al. First-in-human phase 1/2a study of a potent and novel CDK2-selective inhibitor PF-07104091 in patients (pts) with advanced solid tumors, enriched for CDK4/6 inhibitor resistant HR+/HER2- breast cancer. (Abstract). Journal of Clinical Oncology. 2023;41(16):3010.
227. Yap TA, Basu C, Goldman JW, Gordon M, Hamilton E, Kelly A, et al. Abstract P5-16-06: A first-in-human phase 1/2a dose escalation/expansion study of the first-in-class CDK2/4/6 inhibitor PF-06873600 alone or with endocrine therapy in patients with breast or ovarian cancer. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2022.
228. André F, Ciruelos EM, Juric D, Loibl S, Campone M, Mayer IA, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol. 2021;32(2):208-17.
229. Rugo HS, Lerebours F, Ciruelos E, Drullinsky P, Ruiz-Borrego M, Neven P, et al. Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. Lancet Oncol. 2021;22(4):489-98.

230. Almekinders CAM, Konings IRHM, van der Noort V, Berg vd, S.M., Bos MEMM, et al. Abstract OT2-01-08: SEQUence of Endocrine therapy in advanced Luminal Breast cancer (SEQUEL-Breast): A phase 2 study on fulvestrant beyond progression in combination with alpelisib for PIK3CA-mutated, HR+ HER2- advanced breast cancer. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
231. De Laurentiis M, Costa L, Gligorov J, Knop A, Senkus-Konefka E, García-Sáenz JA, et al. EPIK-B5: A phase III, randomized study of alpelisib (ALP) plus fulvestrant (FUL) in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-), PIK3CA-mutated advanced breast cancer (ABC) progressing on/after an aromatase inhibitor (AI) with a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i). Journal of Clinical Oncology. 2022;40(16).
232. Howell SJ, Casbard A, Carucci M, Ingarfield K, Butler R, Morgan S, et al. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive, HER2-negative breast cancer (FAKTION): overall survival, updated progression-free survival, and expanded biomarker analysis from a randomised, phase 2 trial. Lancet Oncol. 2022;23(7):851-64.
233. Turner N, Oliveira M, Howell SJ, Dalenc F, Cortes J, Gomez H, et al. Abstract GS3-04: Capivasertib and fulvestrant for patients with aromatase inhibitor-resistant hormone receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer: Results from the phase III CAPItello-291 trial. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
234. Cook MM, Al Rabadi L, Kaempf AJ, Saraceni MM, Savin MA, Mitri ZI. Everolimus Plus Exemestane Treatment in Patients with Metastatic Hormone Receptor-Positive Breast Cancer Previously Treated with CDK4/6 Inhibitor Therapy. Oncologist. 2021;26(2):101-6.
235. Bardia A, Hurvitz SA, DeMichele A, Clark AS, Zelnak A, Yardley DA, et al. Phase I/II Trial of Exemestane, Ribociclib, and Everolimus in Women with HR. Clin Cancer Res. 2021;27(15):4177-85.
236. Wesolowski R, Rugo H, Stringer-Reasor E, Han HS, Specht JM, Dees EC, et al. Abstract PD13-05: PD13-05 Updated results of a Phase 1b study of gedatolisib plus palbociclib and endocrine therapy in women with hormone receptor positive advanced breast cancer: Subgroup analysis by PIK3CA mutation status. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
237. Rugo HS, Schiavon G, Grinsted LM, De Bruin EC, Catanese MT, Hamilton E. Abstract OT2-14-01: CAPItello-292: A phase Ib/III study of capivasertib, palbociclib and fulvestrant, versus placebo, palbociclib and fulvestrant, for endocrine therapy-resistant HR+/HER2− advanced breast cancer. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2022.
238. Hurvitz SA, Andre F, Cristofanilli M, Curigliano G, Giordano A, Han HS, et al. A phase 3 study of gedatolisib plus fulvestrant with and without palbociclib in patients with HR+/ HER2- advanced breast cancer previously treated with a CDK4/6 inhibitor plus a nonsteroidal aromatase inhibitor (VIKTORIA-1). (Abstract). Journal of Clinical Oncology. 2023;41(16).
239. Manohar PM, Davidson NE. Updates in endocrine therapy for metastatic breast cancer. Cancer Biol Med. 2021;19(2):202-12.
240. Oliveira M, Pominchuck D, Nowecki Z, Hamilton E, Kulyaba Y, Andabekov T, et al. Abstract 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. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
241. Bidard FC, Kaklamani VG, Neven P, Streich G, Montero AJ, Forget F, 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. 2022;40(28):3246-56.
242. Martin Jimenez M, Lim E, Chavez Mac Gregor M, Bardia A, Wu J, Zhang Q, et al. Abstract 211MO: Giredestrant (GDC-9545) vs physician choice of endocrine monotherapy (PCET) in patients (pts) with ER+, HER2– locally advanced/metastatic breast cancer (LA/mBC): Primary analysis of the phase II, randomised, open-label acelERA BC study.: Annals of Oncology; 2022. p. S633-S4.
243. Tolaney SM, Chan A, Petrakova K, Delaloge S, Campone M, Iwata H, et al. AMEERA-3: Randomized Phase II Study of Amcenestrant (Oral Selective Estrogen Receptor Degrader) Versus Standard Endocrine Monotherapy in Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer. J Clin Oncol. 2023:JCO2202746.
244. Hoy SM. Elacestrant: First Approval. Drugs. 2023;83(6):555-61.
245. Downton T, Zhou F, Segara D, Jeselsohn R, Lim E. Oral Selective Estrogen Receptor Degraders (SERDs) in Breast Cancer: Advances, Challenges, and Current Status. Drug Des Devel Ther. 2022;16:2933-48.
246. Dudek AZ, Liu LC, Fischer JH, Wiley EL, Sachdev JC, Bleeker J, et al. Phase 1 study of TTC-352 in patients with metastatic breast cancer progressing on endocrine and CDK4/6 inhibitor therapy. Breast Cancer Res Treat. 2020;183(3):617-27.
247. Johnston S, Pluard TJ, Wang JS, Hamilton EP, Juric D, Scholz CR, et al. Abstract P1-17-03: H3B-6545 in combination with palbociclib in women with metastatic estrogen receptor-positive (ER+), human epidermal growth factor receptor 2 (HER2)-negative breast cancer, phase 1b study. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2022.
248. Patel M, Alemany C, Mitri Z, Makower D, Borges V, Sparano J, et al. Abstract P1-17-12: Preliminary data from a phase I/II, multicenter, dose escalation study of OP-1250, an oral CERAN/SERD, in subjects with advanced and/or metastatic estrogen receptor (ER)-positive, HER2-negative breast cancer. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2022.
249. Schott AF, Hurvitz S, Ma C, Hamilton E, Nanda R, Zahrah G, et al. Abstract 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. San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
250. Damodaran S, Plourde PV, Moore HCF, Anderson IC, Portman DJ. Open-label, phase 2, multicenter study of lasofoxifene (LAS) combined with abemaciclib (Abema) for treating pre- and postmenopausal women with locally advanced or metastatic ER+/HER2− breast cancer and an ESR1 mutation after progression on prior therapies. (Abstract). Journal of Clinical Oncology. 2022;40(16).
251. Hickey TE, Selth LA, Chia KM, Laven-Law G, Milioli HH, Roden D, et al. The androgen receptor is a tumor suppressor in estrogen receptor-positive breast cancer. Nat Med. 2021;27(2):310-20.
252. Panet-Raymond V, Gottlieb B, Beitel LK, Pinsky L, Trifiro MA. Interactions between androgen and estrogen receptors and the effects on their transactivational properties. Mol Cell Endocrinol. 2000;167(1-2):139-50.
253. Fu Z, Li S, Han S, Shi C, Zhang Y. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Signal Transduct Target Ther. 2022;7(1):93.
254. Dumontet C, Reichert JM, Senter PD, Lambert JM, Beck A. Antibody-drug conjugates come of age in oncology. Nat Rev Drug Discov. 2023;22(8):641-61.
255. Grinda T, Rassy E, Pistilli B. Antibody-Drug Conjugate Revolution in Breast Cancer: The Road Ahead. Curr Treat Options Oncol. 2023;24(5):442-65.

256. Abbas AJ, Ibrahim MF, Saifo MS. Antibody-Drug Conjugates Used in Breast Cancers. J Oncol. 2021;2021:9927433.
257. Subhan MA, Torchilin VP. Advances in Targeted Therapy of Breast Cancer with Antibody-Drug Conjugate. Pharmaceutics. 2023;15(4).
258. Staudacher AH, Brown MP. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required? Br J Cancer. 2017;117(12):1736-42.
259. Cortés J, Kim SB, Chung WP, Im SA, Park YH, Hegg R, et al. Trastuzumab Deruxtecan versus Trastuzumab Emtansine for Breast Cancer. N Engl J Med. 2022;386(12):1143-54.
260. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med. 2022;387(1):9-20.
261. Syed YY. Sacituzumab Govitecan: First Approval. Drugs. 2020;80(10):1019-25.
262. Vidula N, Yau C, Rugo H. Trophoblast Cell Surface Antigen 2 gene (TACSTD2) expression in primary breast cancer. Breast Cancer Res Treat. 2022;194(3):569-75.
263. Rugo H, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, et al. Abstract GS1-11: Sacitizumab govitecan (SG) vs treatment of physician's choice (TPC): Efficacy by trop-2 expression in the TROPiCS-02 study of patients (pts) with HR+/HER2- metastatic breast cancer (mBC). San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
264. Rugo HS, Bardia A, Marmé F, Cortes J, Schmid P, Loirat D, et al. Sacituzumab Govitecan in Hormone Receptor-Positive/Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer. J Clin Oncol. 2022;40(29):3365-76.
265. Rugo HS, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, et al. Abstract LBA76: Overall survival (OS) results from the phase III TROPiCS-02 study of sacituzumab govitecan (SG) vs treatment of physician's choice (TPC) in patients (pts) with HR+/HER2- metastatic breast cancer (mBC). European Society for Medical Oncology (ESMO): Annals of Oncology; 2022.
266. Meric-Bernstam F, Krop I, Juric D, Kogawa T, Hamilton A, Spira AI, et al. Abstract PD13-08: Phase 1 TROPION-PanTumor01 study evaluating datopotamab deruxtecan (Dato-DXd) in unresectable or metastatic hormone receptor-positive/HER2-negative breast cancer (BC). San Antonio Breast Cancer Symposium; San Antonio, Texas, USA: Cancer Research; 2023.
267. Bardia A, Jhaveri K, Kalinsky K, Pernas S, Tsurutani J, Xu B, et al. TROPION-Breast01: Datopotamab deruxtecan vs chemotherapy in pre-treated inoperable or metastatic HR+/HER2- breast cancer. Future Oncol. 2023.

FIGURE LEGENDS

Figure 1. Regular cyclin D-CDK4/6 activity mediates molecular events governing cell-cycle progression through G1 phase in normal breast cells. In breast cells, cell cycle outset is driven by proteins in the PI3K signaling pathway and estrogen receptor (ER) signaling pathway, leading to activation of cyclin D/CDK4 and cyclin D/CDK6. Cyclin D-CDK4/6 accumulation allows entry into the cell cycle (G1 phase) thereby avoiding cell cycle exit. Accumulation of cyclin E in this phase leads to an increased activity of cyclin E/CDK2, which inactivates Rb by hyperphosphorylation, and in turn activates E2F-dependent transcription. Hence, E2F-dependent transcription determines accumulation of cyclin E and cyclin A in G1 phase, promoting a positive feedback loop that enhances cyclin E/CDK2 activity, accumulates cyclin A/CDK2 activity, and consequently results in replication initiation at the RICP. In G2 phase, accumulation of cyclin A/CDK1 complex and cyclin B/CDK1 complex promotes mitotic entry at the MICP. In M phase, increase of cyclin A/CDK1 and cyclin B/CDK1 activity complete the cell cycle. Accumulation of cyclin D-CDK4/6 activity then prompts cells

to re-enter the cell cycle. Complete cyclin/CDK activation is induced by CAK phosphorylation throughout the cell cycle. Direct activation (phosphorylation) is shown with arrowhead lines, whereas inhibition (phosphorylation) is indicated with blocked lines. Colored circle lines in the cell cycle show CDK activity, whereas colored dash-dotted cycle lines in the cell cycle indicate CDK inactivity. RTK: receptor tyrosine kinases; PI3K STP: PI3K/AKT/mTORC signal transduction pathway; ER STP: estrogen receptor signal transduction pathway. CDK: cyclin-dependent kinase. E2F: activating E2Fs (E2F1-E2F3); CAK: CDK-activating kinase; RICP: replication initiation commitment point; MICP: mitosis initiation commitment point; DP: dimerization partner transcription factor; Circled P (black background with white text): phosphoryl group during direct or indirect phosphorylation.

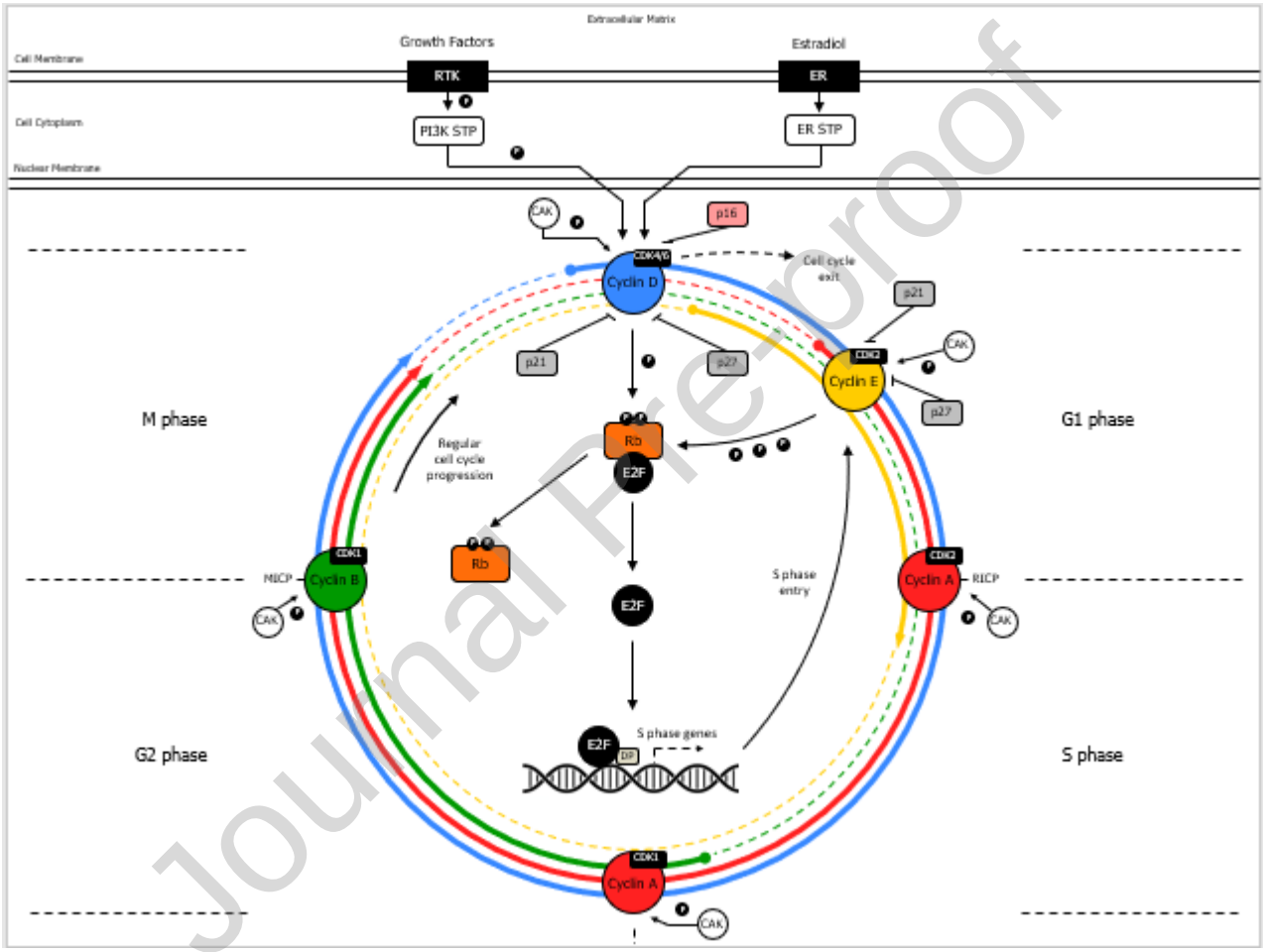

Figure 2. Abnormal cyclin D-CDK4/6 activity mediates molecular events regulating cell-cycle progression through G1 phase in breast cancer cells. In breast cancer cells persistent cell cycle progression is mainly driven by mutations of proteins in PI3K signaling pathway and estrogen receptor (ER), as well as in cyclin D/CDK4 and cyclin D/CDK6. Mutations in pocket protein family (Rb, p107, and p130) enhance E2F dependent transcriptional regulation, thereby endorsing cyclin E/CDK2, as well as downstream cyclin A/CDK2 activity in cancer cells. Mutation-induced CDK overactivity results in augmented phosphorylation and inactivation of Rb, allowing expression of E2F-dependent genes (e.g. cyclin E1-encoding *CCNE1* and cyclin E2-encoding *CCNE2*). Successively, cyclin E binds and activates CDK2, determining further hyperphosphorylation of Rb and phosphorylation of several other molecules, preventing cell cycle exit, and consequently leading to a permanent

commitment to S phase entry. Red lightning symbols show common mutations normally found in signaling pathways upstream cell cycle or related to cell cycle regulation in breast cancer. Direct activation (phosphorylation) is shown with arrowhead lines, whereas inhibition (phosphorylation) is indicated with blocked lines. Colored circle lines in the cell cycle show CDK activity, whereas colored dash-dotted cycle lines in the cell cycle indicate CDK inactivity. Red crosses emphasize signaling blockage in breast cancer, whereas green dash-dotted lines (adjacent to arrowhead lines) highlight signaling enhancement in breast cancer. RTK: receptor tyrosine kinases; PI3K STP: PI3K/AKT/mTORC signal transduction pathway; ER STP: estrogen receptor signal transduction pathway. CDK: cyclin-dependent kinase. E2F: activating E2Fs (E2F1-E2F3); CAK: CDK-activating kinase; RICP: replication initiation commitment point; MICP: mitosis initiation commitment point; DP: dimerization partner transcription factor; Circled P (black background with white text): phosphoryl group during direct or indirect phosphorylation.

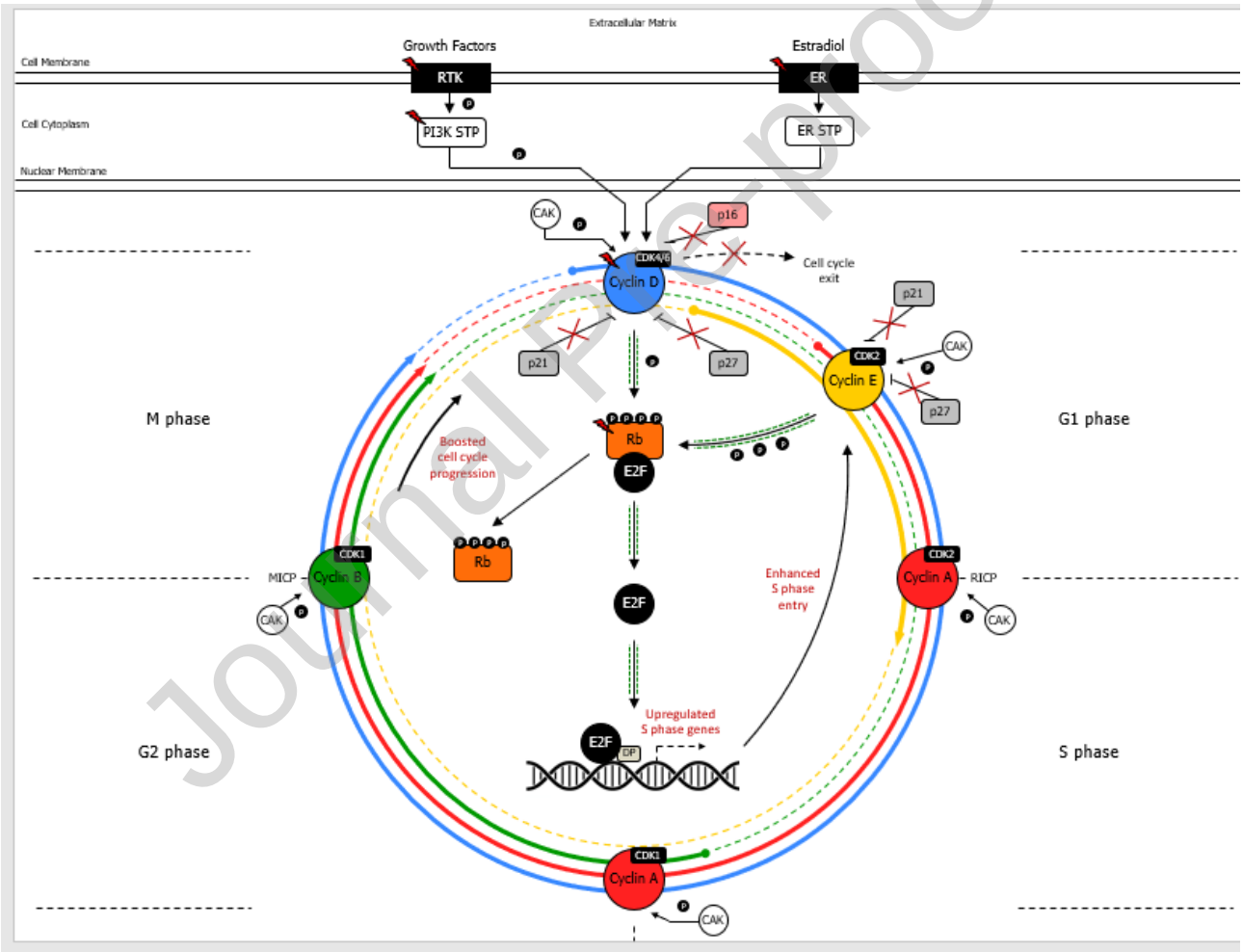

Figure 3. CDK4/6i-mediated inhibition of G1 phase/S phase CDKs. A. The classical model indicates that CDK4/6i suppress active cyclin D-CDK4/6i-p21/p27 trimer holoenzymes, averting CDK4/6-mediated Rb phosphorylation, and blocking S phase entry in the cell cycle. **B**. The Guiley *et al* model suggests that CDK4/6i bind to monomeric CDK4/6, precluding the establishment of cyclin D-CDK4/6-p21/p27 trimer holoenzymes. The resulting freed p21 protein then binds to and suppresses cyclin E/CDK2 complex, preventing Rb phosphorylation,

and blocking S phase entry in the cell cycle. This model indicates that CDK4/6i determine cell cycle arrest through indirect inhibition of CDK2, instead of direct inhibition of CDK4/6 activity. **C**. The Pack *et al* model proposes that CDK4/6i directly inhibit CDK4/6 catalytic activity, but also sequester p21 from already-formed cyclin D-CDK4-p21 trimer holoenzymes, thereby leading to indirect suppression of cyclin E/CDK2 complex, and blocking S phase entry in the cell cycle. Inhibition is indicated with blocked lines. Protein interaction is shown with dotted arrowhead lines. Red crosses emphasize blocked phosphorylation, suppressed gene expression, and G1 phase/S phase arrest of cell cycle in breast cancer. CDK4/6: cyclin-dependent kinase 4 and cyclin-dependent kinase 6; CDK4/6i: cyclin-dependent kinase 4 and cyclin-dependent kinase 6 inhibitors; CDK4: cyclin-dependent kinase 4; CDK2: cyclin-dependent kinase 2; p21*: p21 protein or p27 protein; E2F: activating E2Fs (E2F1-E2F3); DP: dimerization partner transcription factor; circled P (black background with white text): phosphoryl group during phosphorylation.

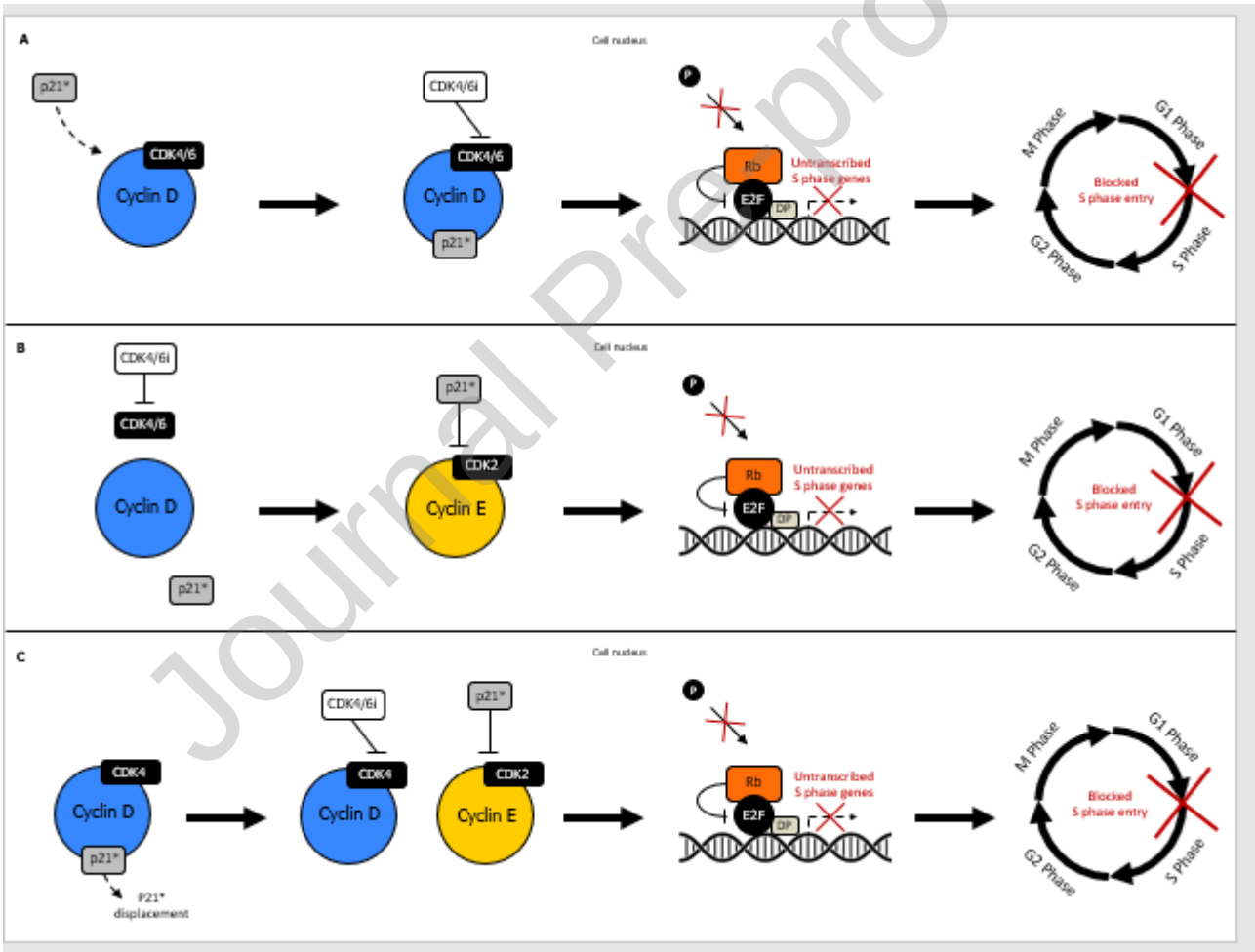

Figure 4. CDK4/6i suppress cell cycle progression to S phase in ER⁺ breast cancer. FDA-approved CDK4/6i palbociclib, ribociclib and abemaciclib, as well as other CDK4/6i currently being tested such as dalpiciclib (phase 3), birociclib (phase 3), FCN-437C (phase 3), TQB3616 (phase 3), PF-06873600 (phase 2), lerociclib (phase 2), BPI-1178 (phase 2), and TY-302 (phase 1), suppress cell cycle progression to S phase in ER⁺ breast cancer. Activation is shown with arrowhead lines, whereas inhibition is indicated with blocked lines. Dotted lines indicate lower relative inhibition activity. Red crosses emphasize signaling

blockage in breast cancer. PI3K STP: PI3K/AKT/mTORC signal transduction pathway; ER STP: estrogen receptor signal transduction pathway; CDK4/6: cyclin-dependent kinase 4 and cyclin-dependent kinase 6; CDK4/6i: cyclin-dependent kinase 4 and cyclin-dependent kinase 6 inhibitors; CDK2: cyclin-dependent kinase 2; CDK9: cyclin-dependent kinase 9; E2F: activating E2Fs (E2F1-E2F3); CAK: CDK-activating kinase; DP: dimerization partner transcription factor; Circled P (black background with white text): phosphoryl group during direct or indirect phosphorylation.

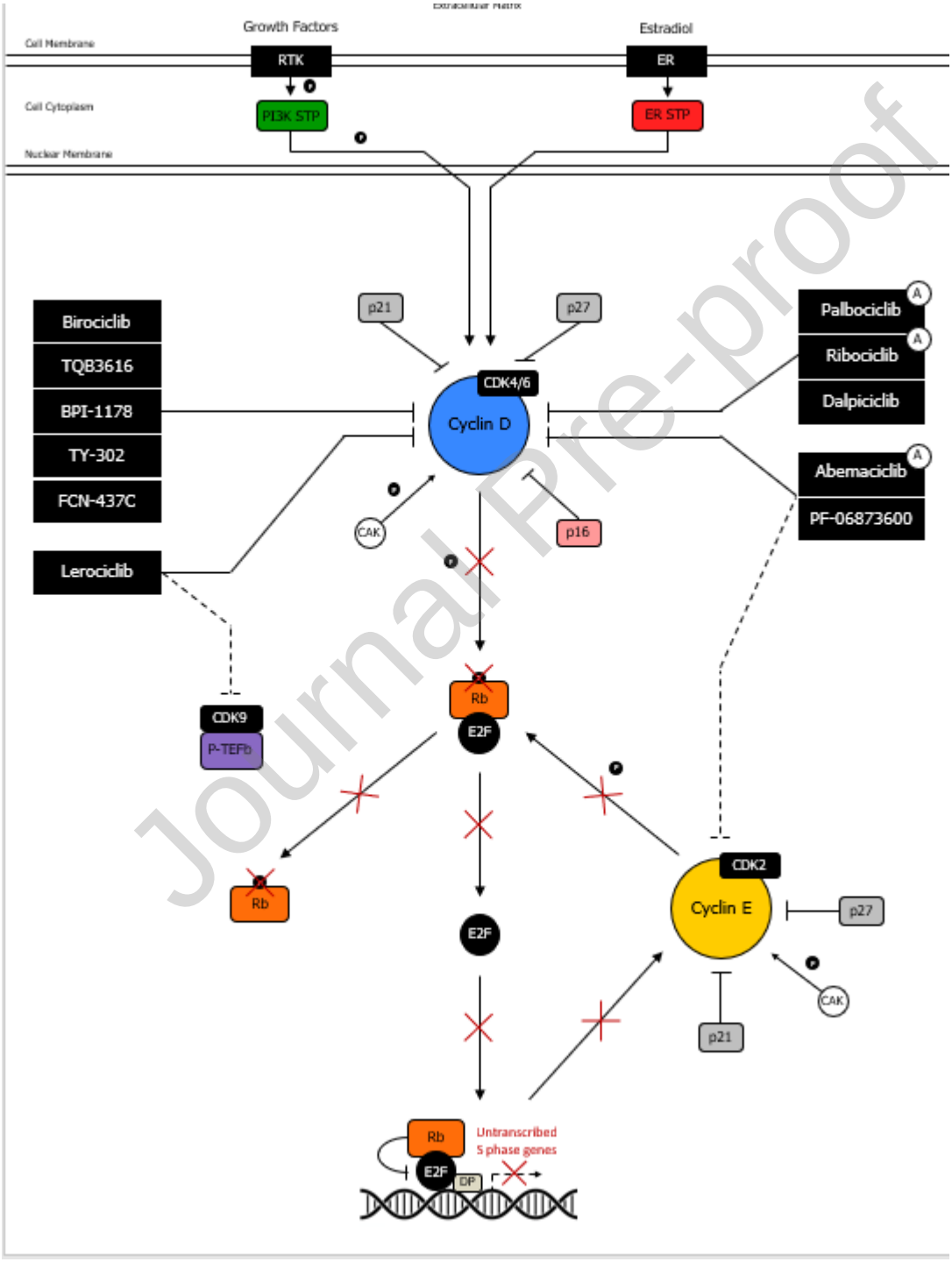

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Figure 5. Mechanisms of CDK4/6i and ET resistance. Mechanisms of CDK4/6i and ET resistance presently reported in literature comprise loss of Rb function, acquired CDK6 amplification, abnormal cyclin E/CDK2 activity, oncogene c-Myc alteration, and activation of PI3K/AKT/mTOR signaling pathway. Activation is shown with arrowhead lines, whereas inhibition is indicated with blocked lines. CDK4/6: cyclin-dependent kinase 4 and cyclin-dependent kinase 6; CDK2: cyclin-dependent kinase 2; ERα: estrogen receptor alpha; E2: estradiol; E2F: activating E2Fs (E2F1-E2F3); CAK: CDK-activating kinase; DP: dimerization partner transcription factor; circled P (black background with white text): phosphoryl group during direct or indirect phosphorylation.

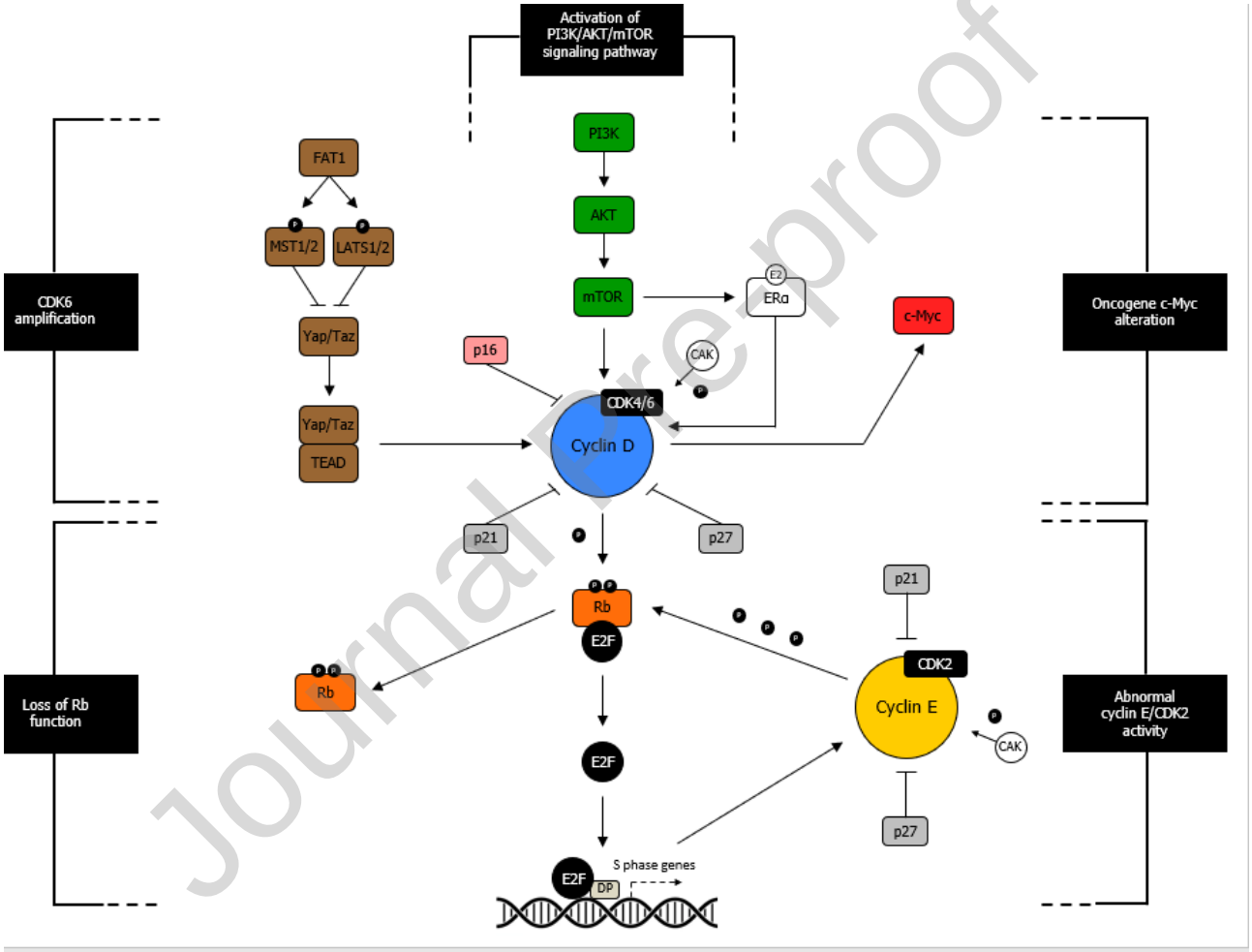

Figure 6. CDK4/6 inhibition induces adaptive rewiring of PI3K/AKT/mTOR signaling pathway. Suppression of CDK4/6 inhibits Rb phosphorylation, thereby impeding E2F-induced cell cycle progression. However, other mechanisms may counterbalance these cell cycle-promoting effects. Indeed, *a)* reduced CDK4/6 activity can downregulate mTORC1 signalling via increased TSC activity [1], which may result to loss of negative feedback regulation of RTK signalling [2], leading to upregulated upstream PI3K/AKT signaling pathway activity and cell proliferation [3]. Similarly, *b)* decreased phosphorylated-Rb levels can also result in augmented AKT phosphorylation by mTORC2 [4], which stimulates cell survival mechanisms. Besides, *c)* compensatory CDK2-mediated Rb phosphorylation [5] can induce cell cycle progression in the absence of CDK4/6 signalling. In PI3K/AKT/mTORC signaling pathway, proteins involved in negative feedback regulation are figured in red

rectangles, whereas proteins involved in positive feedback regulation are figured in green rectangles. Activation (phosphorylation or non-phosphorylation) is shown with arrowhead lines, dephosphorylation is indicated with roundhead lines, and inhibition is displayed with blocked lines. CDK4/6i-mediated adaptive rewiring of PI3K/AKT/mTOR signaling pathway and cyclin E/CDK2 is shown with blocked dotted lines. Red crosses emphasise signaling blockage, whereas green dash-dotted lines (adjacent to arrowhead lines or blocked lines) highlight signaling enhancement. RTK: receptor Receptor tyrosine kinase; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: Phosphatidylinositol (3,4,5)-trisphosphate; TSC: Tuberous sclerosis complex; RHEB: Ras homolog enriched in brain; GDP: Guanosine diphosphate: GTP: Guanosine triphosphate; Rag A and Rag C: Rag heterodimers; CDK4/6: cyclin-dependent kinase 4 and cyclin-dependent kinase 6; CDK4/6i: cyclin-dependent kinase 4 and cyclin-dependent kinase 6 inhibitors; CDK2: cyclin-dependent kinase 2; E2F: activating E2Fs (E2F1-E2F3); circled P (black background with white text): phosphoryl group.

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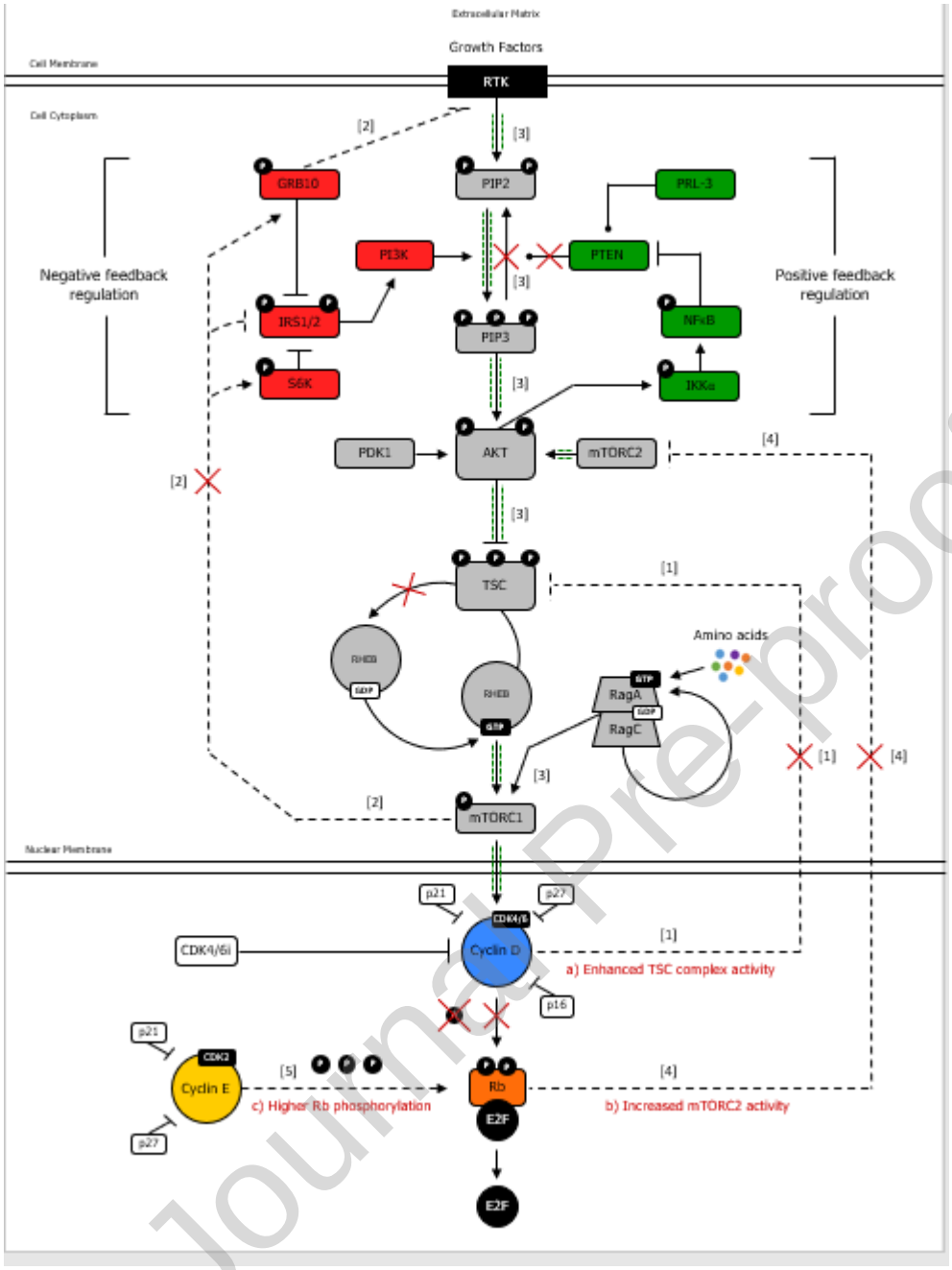

Supplementary Figure 1. Timeline of FDA approvals for small-molecule CDK4/6i palbociclib, ribociclib and abemaciclib in advanced ER+/HER2- BC patients. Small-molecule CDK4/6i palbociclib, ribociclib and abemaciclib are indicated with blue, green and red rectangles, respectively. Black arrows below CDK4/6i: years in which FDA approval of CDK4/6i occurred for a particular type of treatment. Grey squares at the top-left of each CDK4/6i: type/s of treatment related to the FDA approval of the corresponding inhibitor (A: abemaciclib monotherapy; A + F: abemaciclib plus fulvestrant; A + AI: abemaciclib plus aromatase inhibitor; A + ET: abemaciclib plus endocrine thearpy; P + F: palbociclib plus fulvestrant; A + AI: palbociclib plus aromatase inhibitor; R + F: ribociclib plus fulvestrant; R + AI: ribociclib plus aromatase inhibitor). Circled capital letters at the top-right of each CDK4/6i: biopharmaceutical companies related to the FDA approval for a particular type of treatment. Ⓟ: Pfizer; Ⓝ: Novartis; Ⓔ: Eli Lilly.

TABLE LEGENDS

Table 1. Small-molecule CDK4/6i FDA-approved or currently under clinical development used for advanced ER$^+$/HER2$^-$ BC patients. BC: breast cancer; FDA: food and drug administration; IC50: half-maximal inhibitory concentration; Ki: inhibitor constant; N/A: not available; nM: nanomolar; R: reference; (*): not applicable.

Agent	Status	CDK Selectivity (IC50) [Ki]					Clinical development	Company
Palbociclib	Approved	*	*	CDK4 (11 nM)	CDK6 (16 nM)	*	FDA-approved for HR$^+$/HER2$^-$ advanced BC in combination with hormonal therapy	Pfizer
Ribociclib	Approved	*	*	CDK4 (10 nM)	CDK6 (39 nM)	*	FDA-approved for HR$^+$/HER2$^-$ advanced BC in combination with hormonal therapy	Novartis
Abemaciclib	Approved	CDK1 (N/A)	CDK2 (N/A)	CDK4 (2 nM)	CDK6 (10 nM)	CDK9 (N/A)	FDA-approved for HR$^+$/HER2$^-$ advanced BC as monotherapy. FDA-approved for HR$^+$/HER2$^-$ advanced BC in combination with hormonal therapy. FDA-approved for HR$^+$/HER2$^-$ high-risk, early-stage BC in combination with hormonal therapy	Eli Lilly
Dalpiciclib	Phase 3	*	*	CDK4 (12 nM)	CDK6 (10 nM)	*	Phase 3 clinical trial in combination with hormonal therapy in HR$^+$/HER2$^-$ advanced BC (NCT03966898)	Jiangsu Hengrui Medicine
Birociclib	Phase 3	*	*	N/A	N/A	*	Phase 3 clinical trial in combination with fulvestrant in HR$^+$/HER2$^-$ advanced BC (NCT05077449)	Xuanzhu Biopharmaceutical
FCN-437C	Phase 3	*	*	N/A	N/A	*	Phase 3 clinical trial in combination with fluvestrant ± goseraline in HR$^+$/HER2$^-$ advanced BC (NCT05438810)	Ahon Pharmaceutical
TQB3616	Phase 3	*	*	N/A	N/A	*	Phase 3 clinical trial in combination with ET (letrozole or anastrozole or tamoxifen) in HR$^+$/HER2$^-$ advanced BC (NCT05780567). Phase 3 clinical trial in combination with fulvestrant in HR$^+$/HER2$^-$ advanced BC (NCT05375461)	Chia Tai Tianqing Pharmaceutical

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PF-06873600	Phase 2	*	CDK2 (0.09 nM) [Ki]	CDK4 (0.13 nM) [Ki]	CDK6 (0.16 nM) [Ki]	*	Phase 2 clinical trial as monotherapy or in combination with hormonal therapy in $HR^+/HER2^-$ metastatic BC (NCT03519178)	Pfizer
Lerociclib	Phase 2	*	*	CDK4 (1 nM)	CDK6 (2 nM)	CDK9 (28 nM)	Phase 2 clinical trial in combination with fulvestrant in $HR^+/HER2^-$ advanced or metastatic BC (NCT05085002)	EQRx International
BPI-1178	Phase 2	*	*	N/A	N/A	*	Phase 2 clinical trial in combination with standard ET (fulvestrant or letrozole) in $HR^+/HER2^-$ advanced BC (NCT04282031)	Beta Pharma
TY-302	Phase 1	*	*	N/A	N/A	*	Phase 1 clinical trial as monotherapy or in combination with tamoxifen in $HR^+/HER2^-$ advanced BC (NCT04433494)	TYK Medicines

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Supplementary Table 1. Clinical use of FDA-approved small-molecule CDK4/6i palbociclib, ribociclib and abemaciclib in advanced ER+/HER2- BC patients. MRA: medication routes of administration; AI: aromatase inhibitor; BC: breast cancer; ET: endocrine therapy.

Supplementary Table 2. Completed and ongoing clinical trials with favorable, acceptable or manageable safety profile, which have already shown results in ER$^+$/HER2$^-$ BC patients after progression on small-molecule CDK4/6i in advanced ER$^+$/HER2$^-$ BC patients. ADCs: antibody drug conjugates; AKTi: AKT inhibitor/s; AI: aromatase inhibitor; allosteric non-competitive mTORi: allosteric non-competitive mTOR inhibitor/s; A/NR: active, not recruiting; A/R: active, recruiting; C: completed; CA: comparator arm; CBR: clinical benefit rate; CDK4/6i: CDK4/CDK6 inhibitor/s; CERAN: complete estrogen receptor antagonist; dual PI3K/mTORi: dual PI3K/mTOR inhibitor/s; ET: endocrine therapy; FP/LUP: first posted/last update posted; IA: investigational arm; I/D: intermittent dosing; N/A: not applicable; NCT: national clinical trial; №/P: number of ER$^+$/HER2$^-$ BC patients; N/R: not reported; ORR: overall response rate; OS: overall survival; PCI: Prior CDK4/6i (%); PFS: Progression-free survival; PROTAC: proteolysis targeting chimera; SERCA: selective estrogen receptor covalent antagonist; SERD: selective estrogen receptor degrader; SERM: selective estrogen receptor modulator; ShERPA: selective human estrogen receptor (ER) partial agonist; W/D: weekly dosing; (*): № of patients in the ER$^+$/HER2$^-$ BC cohort.

Supplementary Table 3. Ongoing clinical trials which have not yet displayed results in ER$^+$/HER2$^-$ BC patients following progression on CDK4/6i. ADCs: antibody drug conjugates; AI: aromatase inhibitor; Allosteric non-competitive mTORi: allosteric non-competitive mTOR inhibitor/s; ASTs: advanced solid tumors; BC: breast cancer; CDK2i: CDK2 inhibitor/s; CDK4/6i: CDK4/CDK6 inhibitor/s; ET: endocrine therapy; ICIs: immune checkpoint inhibitors; MTD: maximum tolerated dose; N/A: not applicable; NCT: national clinical trial; №/P: number of ER$^+$/HER2$^-$ BC patients; ORR: overall response rate; OS: overall survival; PFS: Progression-free survival; PROTAC: proteolysis targeting chimera; RP2D: recommended phase 2 dose; RP3D: recommended phase 3 dose; SCE: Study Completion (Estimated); SERD: selective estrogen receptor degrader. (*): Anticipated total study enrolment of clinical trial still recruiting ER$^+$/HER2$^-$ BC patients.

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Declaration of interest

S.A.W.: Consulting/Advisory Board: Foundation Medicine, Eli Lilly, Novartis, Astrazeneca, Biovica, Hologic, Pfizer, Puma Biotechnology; Education/Speaking: Guardant Health, Eli Lilly, 2ndMD; Institutional Research Support: Genentech, Eli Lilly, Pfizer, Pfizer, Nuvation Bio, Regor Therapeutics. A.B.: Consultant/Advisory board of: Pfizer, Novartis, Genentech, Merck, Radius Health, Immunomedics/Gilead, Sanofi, Daiichi Pharma/Astra Zeneca, Phillips, Eli Lilly, Foundation Medicine. Contracted Research/Grant (to institution): Genentech, Novartis, Pfizer, Merck, Sanofi, Radius Health, Immunomedics/Gilead, Daiichi Pharma/Astra Zeneca, Eli Lilly.

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Highlights of the review article "*Mechanisms of sentitivity and resistance to CDK4/CDK6 inhibitors in hormone receptor-positive breast cancer treatment*"

• Cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) are key molecules in the G1-to-S phase cell cycle transition and are crucial for the onset, survival, and progression of breast cancer (BC). In this review we describe how small-molecule CDK4/CDK6 inhibitors (CDK4/CDK6i) block phosphorylation of tumor suppressor Rb and thus restrain susceptible BC cells in G1 phase.

• Three CDK4/CDK6i have been FDA-approved for the first-line treatment of patients with advanced/metastatic hormone receptor-positive (HR^+)/human epidermal growth factor receptor 2-negative ($HER2^-$) BC in combination with endocrine therapy (ET): palbociclib (Pfizer), ribociclib (Novartis), and abemaciclib (Eli Lilly). In this review we discuss in detail these three FDA-approved CDK4/CDK6i.

• CDK4/CDK6i can modulate other distinct effects in both BC and breast stromal compartments, which may provide new insights into aspects of their clinical activity. In this review we describe the biochemistry of CDK4/CDK6-Rb-E2F pathway in HR^+ BC and then discuss in detail how CDK4/CDK6i are also involved in triggering other effects in BC/breast stromal compartments.

• Up to now there is still no established standard next-line treatment to tackle drug resistance toward palbociclib, ribociclib, and abemaciclib. In this review we outline the mechanisms of CDK4/CDK6i resistance that have emerged in recent preclinical studies and clinical cohorts, emphasizing the impact of these findings on novel therapeutic opportunities and strategies in BC.

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