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CDK4/6 Inhibition in Breast Cancer: Mechanisms of Response and Treatment Failure

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Abstract

Purpose of review—To describe the role of D-type cyclins and CDKs 4 and 6 in breast cancer, and to discuss potential biomarkers for sensitivity or resistance to CDK4/6 inhibitors.

Recent findings—A small number of preclinical and clinical studies have explored potential mechanisms of CDK4/6 inhibitor response and resistance in breast cancer. Putative markers of response include ER-positivity, luminal patterns of gene expression, high cyclin D1 levels, and low p16 levels. Possible resistance mechanisms include loss of Rb function, overexpression/amplification of cyclin E, and CDK6 amplification. Most these remain speculative and have not been validated in clinical specimens.

Summary—If early successes with CDK4/6 inhibitors are to be capitalized upon, it is critical that our understanding of CDK4/6 biology in breast cancer extends beyond its current rudimentary state. Only then we will be able to develop rational therapeutic combinations that further enhance the efficacy of these agents.

Keywords

breast cancer; CDK4/6; cyclin; drug resistance; estrogen receptor

INTRODUCTION

In order for a healthy cell to divide, it must pass through each stage of the cell cycle in a sequential and tightly orchestrated fashion. The control of cellular proliferation is governed by a vast array of molecular players, most important of which are the cyclins and their partner kinases, the cyclin-dependent kinases (CDKs). Given the fundamental role of dysregulated cellular proliferation in cancers, it is not surprising, therefore, that the

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Conflict of Interest

Ana C. Garrido-Castro declares no conflict of interest.

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development of drugs that inhibit the cyclin/CDK axes in tumor cells has been the subject of investigation for many years.

The cell cycle machinery is frequently dysregulated in cancer through a variety of mechanisms. For example, certain tumors harbor amplification of genes encoding particular cyclins and CDKs, hence increasing their levels within tumor cells(1). In other cases, genes for endogenous CDK inhibitors are deleted, facilitating unconstrained CDK activity(2). Most commonly, activation of upstream mitogenic pathways (e.g. PI3K-AKT-mTOR or Ras-Raf-MEK-ERK) leads to increased levels of particular cyclins(3). The D-type cyclins and their partner kinases, CDKs 4 and 6, play a particularly important role in breast cancer. In mouse models, mammary tumorigenesis often requires the presence of cyclin D1 and CDK4, and both are also needed for the growth of certain established mammary tumors(4–6). As detailed below, the cyclin D-CDK4/6 complexes are critical regulators of cellular transition through the G1 to the S phase of the cell cycle.

Despite the knowledge that cyclin D-CDK4/6 complexes are very important for breast cancer growth, pharmacologic targeting of CDK4 and 6 has remained an elusive goal until recently. Older generations of CDK inhibitors lacked specificity, potency, or both, and yielded disappointing clinical trial results. Recently, three relatively selective inhibitors of CDKs 4 and 6 have entered clinical development (palbociclib, ribociclib, and abemaciclib) (7). Each of these is a potent inhibitor of CDK4 and 6, and abemaciclib may also possess inhibitory activity against other kinases, including CDK9(8). Early phase clinical trials in breast cancer have shown extremely promising results(9, 10) and as a testament to the activity of these agents, they have progressed rapidly to phase 3 trials. One (palbociclib) has received U.S. Food and Drug Administration (FDA) approval as a treatment for advanced, estrogen receptor-positive (ER-positive) breast cancer at the time of writing(11, 12). Although outside the scope of this article, it is worth noting that these three inhibitors show differential relative potency for inhibition of CDK4 and CDK6, as well as distinct pharmacokinetic and toxicity profiles.

For reasons discussed below, the main development path for CDK4/6 inhibitors in breast cancer has been in the setting of ER-positive breast cancer. Other pharmacologic agents have also been studied in this disease subtype, including inhibitors of mTOR(13) and PI3-Kinase (particularly for tumors harboring *PIK3CA* mutations)(14–16). Each has shown evidence of efficacy, testament to the multitude of pathways that serve to mediate resistance to standard endocrine therapy. In this review, we focus on the mechanisms behind CDK4/6 inhibitor efficacy, and also discuss potential biomarkers of both response and resistance to these agents.

CELL CYCLE REGULATION: The role of CDKs 4 and 6 in the G1-S transition

The mammalian cell cycle is composed of the interphase during which DNA is replicated and repaired (G1, S and G2) followed by the mitotic phase in which chromosome segregation and cell division occur (G2 and M)(17). In order for a cell to proceed through the cell cycle from G1 into S phase, it must pass through a “restriction point” which is tightly regulated by the retinoblastoma tumor suppressor protein (Rb)(18). In particular, the

phosphorylation of Rb is a critical trigger for passage through the restriction point (see Figure 1).

Classically, the first event triggering Rb phosphorylation is a proliferative stimulus. Stimulation can arise after exposure to extracellular mitogens and growth factors, or due to dysregulation of proliferative signaling pathways within cancer cells. Collectively, these elevate intracellular D-type cyclin levels (cyclins D1, D2, and D3). The amount of D-type cyclin within the cellular nucleus is controlled at the levels of transcription, nuclear retention, and protein stability, and all are governed by mitogenic signaling(19, 20). Once present, D-type cyclins preferentially associate with CDK4 and CDK6, and the holoenzyme phosphorylates Rb in addition to other Rb family members known as “pocket proteins” (RBL1, also known as p107, and RBL2, also known as p130). This phosphorylation of Rb partially uncouples it from the E2F transcription factors, in turn enabling the expression of E-type cyclins. CDK2-cyclin E complexes then act to further phosphorylate and completely inactivate Rb and the pocket proteins. This ultimately results in a more complete derepression of E2F transcription factor activity, facilitating transcription of genes promoting transition into S phase(21).

The catalytic function of CDKs 4 and 6 is regulated by several mechanisms(22). Their activation is mainly controlled by binding to cyclins, which show a cyclical pattern of synthesis and degradation. CDK4/6 activation also requires a second step – the phosphorylation of the Thr160 residue of the CDK activation loop by CDK-activating kinase (CAK). The Cdc25A phosphatase also assists in CDK4 activation by removing inhibitory phosphate groups from various tyrosine residues. In addition to decreasing levels of D-type cyclins as cells progress through S phase, endogenous inhibition of CDK4/6 is also enabled by two families of CDK inhibitors: the INK4 family ($p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$) and the Cip/Kip family (p21, p27, and p57). The INK4 family is composed of 15–20 kDa proteins with repeated ankyrin motifs that facilitate binding to CDK4 and CDK6 and inhibit the construction of CDK4/6-cyclin D complexes. Notably, INK4A and INK4D require the presence of functional Rb to induce cell cycle arrest, as demonstrated by the lack of growth arrest observed with overexpression of $p16^{INK4A}$ and $p19^{INK4D}$ in Rb-deficient cells(23). On the other hand, Cip/Kip family members bind to all cell cycle-related CDKs and have more complex positive or negative regulatory functions. Of note, p21 and p27 can bind to cyclin D-CDK4 complexes in G1, stabilizing these complexes. Their sequestration in these complexes, in turn, relieves inhibition cyclin E-CDK2 complexes(19, 22, 24).

THE ROLE OF CYCLIN D-CDK4/6 IN BREAST CANCER

Given the role that D-type cyclins and CDKs 4 and 6 play in regulating cell cycle progression, it is not surprising that aberrant upregulation of their activity is a common feature in cancer(25). Notably, the cyclin D1-CDK4 axis plays a particularly important role in mammary tissue, and in breast cancer. Although mice lacking cyclin D1 are viable and show few organ-specific deficits, they demonstrate specific defects in the development and proliferation of mammary tissue during pregnancy(26). Conversely, transgenic mice engineered to overexpress *CCND1* in the mammary glands demonstrate abnormal mammary proliferation and, in some instances, develop mammary adenocarcinoma (27). Although it

remains unclear as to why cyclin D1/CDK4 are of particular importance in the mammary epithelium, these observations do suggest that mammary epithelial cells might show particular sensitivity to the inhibition of CDK4/6(28).

Preclinical data further supports the notion that cyclin D1 and CDK4 are critical players in some breast cancers(29). In an elegant study, Yu and colleagues demonstrated that cyclin D1 knockout mice are completely resistant to the formation of breast cancers induced either by the *Erbb2* or *Ras* oncogenes(4). This demonstrates a critical role for cyclin D1 in tumor initiation. Notably, cyclin D1 deficiency did not protect against formation of *c-Myc* or *Wnt-1* driven tumors, suggesting that the role of cyclin D1 in mammary carcinoma is pathway-specific. Indeed, additional analyses *in vitro* and *in vivo* demonstrated that *Ras* and *Erbb2* are dependent on cyclin D1 for malignant transformation of mammary epithelial cells and that these oncogenic pathways act through regulatory elements located within *CCND1* promoter, as opposed to *Wnt-1*- and *Myc* that may be connected to the cell cycle machinery of breast tumors through other targets. A subsequent study confirmed that the role of cyclin D1 in this context is dependent upon its capacity to activate CDK4, as genetic ablation of *Cdk4* in *Erbb2* models also protected mice from the development of mammary tumors(5).

Transgenic animal studies also show that cyclin D1/CDK4 are important for the maintained growth of already established mammary tumors. Acute and global genetic ablation of *Ccnd1* in adult female mice halts progression of *Erbb2*-driven mammary carcinomas, accompanied by cessation of cell proliferation and induction of a senescent-like phenotype in tumor cells(6). Similar results are seen after pharmacologic inhibition of CDK4/6 activity with palbociclib or abemaciclib(6, 30). Treatment with CDK4/6 inhibitors in these tumors inhibits Rb phosphorylation, reduces expression of E2F target genes, and reduces expression and phosphorylation of the FOXM1 transcription factor, all of which contribute to a senescent-like state in cancer cells(31).

In addition to this empirical evidence, there is a strong theoretical rationale for the importance of the cyclin D-CDK4/6 axis in breast cancer. This rationale primarily relates to the presence of significant crosstalk between the cell cycle machinery and oncogenic signaling pathways in breast cancer (Figure 1). First, approximately 75% of all breast cancers express ER and show estrogen-dependent growth, which is specifically dependent upon cyclin D1. Estrogenic steroids promote cell cycle progression in G1-arrested MCF-7 cells by increasing *CCND1* transcription, and *CCND1* is indeed a known ER target gene(32). Furthermore, estrogens also induce Cdc25A expression. Finally, cyclin D1 can also directly bind to ERα and induce ER-mediated transcription, even in the absence of estradiol(33).

Second, approximately 15 percent of breast cancers harbor amplification of *ERBB2*, which encodes the transmembrane growth factor receptor HER2, or activating mutations in *PIK3CA* (approximately 35 percent), which encodes for the p110-catalytic subunit of PI3-kinase. Heightened activity of the HER2-PI3K-AKT axis increases cyclin D1 levels in cells through a variety of mechanisms: (i) HER2-EGFR heterodimers can trigger MAP-kinase pathway signaling which directly activates *CCND1* transcription(19, 34–36); (ii) AKT phosphorylates glycogen synthase kinase-3β (GSK-3β) in a site-specific manner, reducing

cyclin D1 phosphorylation and hence preventing its nuclear export and proteosomal degradation(37).

Finally, studies of human breast cancers have revealed genomic aberrations that might be expected to predict increased cyclin D-CDK4/6 activity. Luminal A, luminal B and HER2-enriched tumors often harbor amplification of *CCND1* (29%, 58% and 38%, respectively), and/or CDK4 gain (14%, 25% and 24%, respectively) (38). In addition, luminal cancers (particularly luminal A tumors) show high levels of Rb protein and mRNA expression.

BIOMARKERS OF RESPONSE AND RESISTANCE TO CDK4/6 INHIBITORS

Clinical trials of CDK4/6 inhibitors in breast cancer have, in general, shown very encouraging results. In concordance with their mechanism of action, CDK4/6 inhibitors have resulted in prolongation of stable disease in patients with advanced ER-positive breast cancer, both when given as monotherapy or in combination with endocrine therapy(9, 10, 39). Intriguingly, a small proportion of patients with ER-positive breast cancer have also experienced regression of metastatic disease in response to CDK4/6 inhibitor monotherapy(9, 10, 40). Given that ER-positive breast cancer cells have not been shown to undergo apoptosis in response to CDK4/6 inhibition, the mechanisms behind true tumor responses remain elusive.

Larger randomized trials have confirmed that CDK4/6 inhibitors have significant activity in ER-positive disease, as shown in Table 1(41–46). All such trials have restricted study entry to patients with ER-positive disease, based on preclinical evidence that luminal breast cancer cells (largely ER-positive) retain higher levels of Rb and show greater sensitivity to CDK4/6 inhibition(47). Notably, not all patients in these studies derived benefit from CDK4/6 inhibition. Similarly, there is speculation that breast cancers in other clinical subgroups (e.g. HER2-positive or triple-negative cancers) might also be candidates for therapeutic CDK4/6 inhibition in particular circumstances(30, 48). For these reasons, it is critical to identify biomarkers that accurately predict response and resistance to CDK4/6 inhibitors.

Biomarkers of Response

a. *Estrogen-receptor positivity.* For reasons described above, all randomized clinical trials have explored the use of CDK4/6 inhibitors in patients with ER-positive disease. Although the positive results from these trials do not confirm the utility of ER-positivity as a biomarker for drug efficacy, early phase clinical data does support this notion. For example, in a phase 1 study, abemaciclib monotherapy was administered to 47 patients with metastatic breast cancer, 36 of which were ER-positive(9). All eleven responses were seen in patients with ER-positive tumors. It is worth noting that in an analysis of primary tumor specimens from the randomized PALOMA-2 study (letrozole vs. letrozole plus palbociclib in advanced ER-positive breast cancer), the degree of benefit from palbociclib did not differ by the level of ER-protein expression as measured by immunohistochemistry(49). Similar observations were made in the randomized PALOMA-3 trial that explored fulvestrant in combination with palbociclib in patients with pretreated metastatic disease(43). However, given the biologic

rationale for using these agents in ER-positive tumors (ER-driven cyclin D1 expression and higher Rb levels), it is reasonable at this time to state that ER-positivity is a useful clinical marker to identify potential candidates for CDK4/6 inhibitor therapy. Although FDA approval has only been granted to date in the setting of ER-positive disease, it still remains unclear as to whether this is the only subgroup of tumors that derive benefit from these agents.

b. *Luminal pattern of gene expression.* In a study determining the effects of palbociclib in a large number of human breast cancer cell lines, Finn et al. noted that almost all sensitive cell lines showed a luminal pattern of gene expression. These cell lines also showed higher levels of *RB1* and *CCND1* mRNA compared to the resistant group. Interestingly, some of the sensitive cell lines were ER-negative(47). Based on these data, it is appealing to speculate that human breast cancers with a luminal gene expression pattern (for example, as assessed by the PAM50 gene set)(50) might show sensitivity to CDK4/6 inhibition, irrespective of ER status. This hypothesis remains to be tested in clinical trial samples, however.

c. *CCND1 amplification and/or loss of p16.* Both basic biology and the preclinical data of Finn suggested that tumors with higher levels of cyclin D1 or lower levels of $p16^{INK4a}$ might be more sensitive to CDK4/6 inhibitors(47). These hypotheses have been tested in numerous clinical samples through assessment of gene copy number and protein expression. First, in a phase II study of single-agent palbociclib in 37 patients with Rb-positive breast cancer, neither $p16^{INK4A}$ nuclear expression nor *CCND1* amplification were predictive of clinical benefit or prolonged progression-free survival (10).

These findings have been upheld in two randomized cohorts. In the phase II PALOMA-1 study, 165 postmenopausal women with previously untreated ER-positive, HER2-negative advanced breast cancer were randomized to the combination of letrozole and palbociclib or letrozole alone(39). Two sequential cohorts were designed in an attempt to identify biomarkers of response. In the first cohort, only ER-positivity and HER2-negative status were considered for enrolment. In the second, central confirmation of *CCND1* amplification and/or $p16^{INK4A}$ loss in the tumor specimen was required. Ultimately, patient selection based on *CCND1* amplification or $p16^{INK4A}$ loss, compared to ER/HER2 status alone, did not predict for benefit from palbociclib(39). Similarly, immunohistochemical staining for cyclin D1 and $p16^{INK4A}$ on the PALOMA-2 specimens did not show differential benefit from palbociclib in patients with tumors with different degrees of expression for either of these proteins(49).

Importantly, *CCND1* amplification is only one reason for heightened cyclin D1 levels within tumor cells (as discussed above), and immunohistochemistry can be an imprecise measure. Assessment of *CCND1* mRNA levels within tumors has not been studied as a predictor of CDK4/6 inhibitor benefit, and might provide more meaningful insights than those we currently have.

d. *Tumor proliferative capacity.* In vitro, CDK4/6 inhibition has shown significant activity against breast cancer cell lines with widely varying doubling times, and there is no evidence to suggest that cells with higher or lower proliferation rates show preferential sensitivity to CDK4/6 inhibitors. Consistent with this, primary tumor Ki-67 levels were not predictive of palbociclib benefit in the PALOMA-2 study(49).

Biomarkers of Resistance

a. *Loss of Rb function.* Given that CDK4/6 inhibitors function primarily by suppressing Rb phosphorylation, it would seem intuitive that tumors lacking functional Rb (and thus showing unconstrained E2F transcription factor activity) are resistant to these agents. Indeed cell line experiments suggest that cells with low Rb mRNA levels are less sensitive to CDK4/6 inhibitors(47). A challenge with validating this hypothesis in clinical samples is determining the appropriate method to measure Rb status, and the appropriate "cut-off" that separates Rb-proficient from Rb-deficient tumors. Immunohistochemistry is convenient, but it is not known whether all Rb detected with this method is functional. Furthermore, results vary considerably between labs. Alternate methods include measurement of gene expression signatures of Rb loss-of-function and genomic sequencing to look for *RB1* mutation or gene loss, although the latter may markedly underestimate the frequency of Rb dysfunction.

In the laboratory, chronic loss of Rb has been associated with the development of a CDK4/6-inhibitor resistant state in breast cancer cell lines(51). The same was true in explants derived from human breast tumors(48). Recently, Malorni et al. interrogated data from *The Cancer Genome Atlas* to develop an 87-gene expression signature of Rb loss (RBsig)(52). The RBsig confirmed the previously established notion that tumors with impaired Rb function are associated with worse prognosis. Importantly, the Rbsig was able to predict with reasonable accuracy the likelihood of breast cancer cell lines being sensitive to palbociclib (high RBsig correlated with relative palbociclib resistance)(52).

Notably, the RBsig scores were highest in luminal A breast cancers and lowest in basal like breast cancers, in keeping with the notion that ER-positive tumors in general retain greater Rb functionality(52). This begs the question of whether the most meaningful and accurate biomarker for CDK4/6 inhibitor-resistance might in fact be loss of Rb function rather than ER-negativity. This is an important distinction, as a subgroup of triple-negative breast cancers also retains Rb expression (e.g. luminal androgen receptor positive tumors)(53), and such tumors have been found in the laboratory to be sensitive to CDK4/6 inhibition(53, 54). In addition, although the majority of ER-positive primary breast cancers show Rb expression, the rate of Rb functional loss in the metastatic setting is unknown.

b. *Hyperactivity of the cyclin E-CDK2 axis.* Aside from CDKs 4 and 6, CDK2 is the kinase that also has the capacity to phosphorylate Rb, and the presence of sustained CDK4/6 inhibition, CDK2 can substitute for CDK4/6, providing a mechanism of escape from cell cycle arrest(51). *In vitro* studies in ER-positive

breast cancer cell lines have shown that chronic exposure to palbociclib can lead to sustained expression of CDK2 and/or its binding partner cyclin E. In cell lines, this can be mediated by *CCNE1* amplification, which may play an important role in early adaptation and acquired resistance to palbociclib(55). Interestingly, CDK4/6 inhibition can also increase tumor cell AKT phosphorylation(30, 55), which increases cyclin D1 levels. In this state, cyclin D1 is able to bind to and activate CDK2 in a non-canonical manner(55). Of note, inhibition of the PI3K-AKT-mTOR axis is able to prevent this phenomenon, and heightened CDK2 activity is potentially amenable to pharmacological inhibition, with CDK2 inhibitors currently in early phases of clinical development.

c. *Increased CDK6 activity.* Although mutations in the kinase domains of CDK4 or 6 have not been reported as mechanisms of resistance to CDK4/6 inhibitors, a recent report describes *in vitro* evidence of *CDK6* amplification in breast cancer cell lines with acquired abemaciclib resistance(56). This amplification was sufficient to promote resistance. It is not known as yet whether this phenomenon is observed in human tumors.

d. *Other genomic aberrations (ESR1, PIK3CA, TP53).* Investigators have interrogated clinical trial samples in an attempt to uncover other genomic predictors for resistance to CDK4/6 inhibitors. For example, the PALOMA-3 study was analyzed to determine the predictive role of mutations in the ER gene *ESR1*, which often occur in the setting of resistance to aromatase inhibitor therapy(57, 58). *ESR1* mutations in ctDNA were analyzed in baseline blood samples of 360 patients, and found in approximately 25% of patients. Although an ESR1 mutation was associated with a worse outcome compared to *ESR1* wild-type status in the overall population, *ESR1* mutant and wild-type patients derived similar PFS benefit with palbociclib (HR 0.43 and 0.49, respectively) (59). Similarly, mutations in *PIK3CA* in plasma were also not associated with palbociclib treatment effect(43). Finally, it is notable that in the abemaciclib phase 1 study, *TP53* mutations were more common in non-responding tumors(9). Given the role of TP53 in mediating cellular senescence, there is a biologic rationale behind this observation, but it awaits confirmation in larger randomized datasets.

CONCLUSIONS

Cell cycle regulation through CDK-targeted drugs has revolutionized the treatment of metastatic breast cancer. Dramatic improvements in clinical outcomes have positioned CDK4/6 inhibitors as a new standard of care for patients with ER-positive disease, in combination with endocrine therapies. This has encouraged widespread interest in exploring the mechanisms of response and failure to these drugs, although many questions remain unanswered.

Moving forward, three priorities clearly exist. The first is to interrogate samples from existing randomized studies in more depth. Ideally, trials in metastatic disease should utilize metastatic tissue when ever possible, given its possible discordance with primary archival

tissue. Moreover, a number of biopsy-rich neoadjuvant studies have now been completed, and the tissue from these studies (obtained before and after CDK4/6 inhibitor therapy) is an invaluable resource. Critically, assays performed on clinical samples must be carefully considered before they are performed. For example, consideration must be given to the best way to assess Rb functionality in tumors.

The second priority is to better comprehend the biological mechanisms underlying CDK4/6 inhibitor activity in cancer. It is clear that these effects extend well beyond cell cycle arrest, and include development of a senescent phenotype, changes in tumor cell kinase signaling, and altered metabolism(30, 31, 60). Until these phenomena are better understood, our understanding of response and resistance mechanisms will remain rudimentary.

Third, it is critical that we understand whether CDK4/6 inhibitors show any activity in tumors that have developed “resistance” to these agents. Anecdotal reports from laboratory studies suggest that breast cancers that respond to CDK4/6 inhibitors and subsequently develop resistance might in fact be re-sensitized to these agents after a short drug holiday. This begs the clinical question of whether using CDK4/6 inhibitors beyond progression might be a useful strategy. It is important to understand the biological basis for such observations before large-scale clinical trials, which are expensive and expose patients to drug toxicity, are begun.

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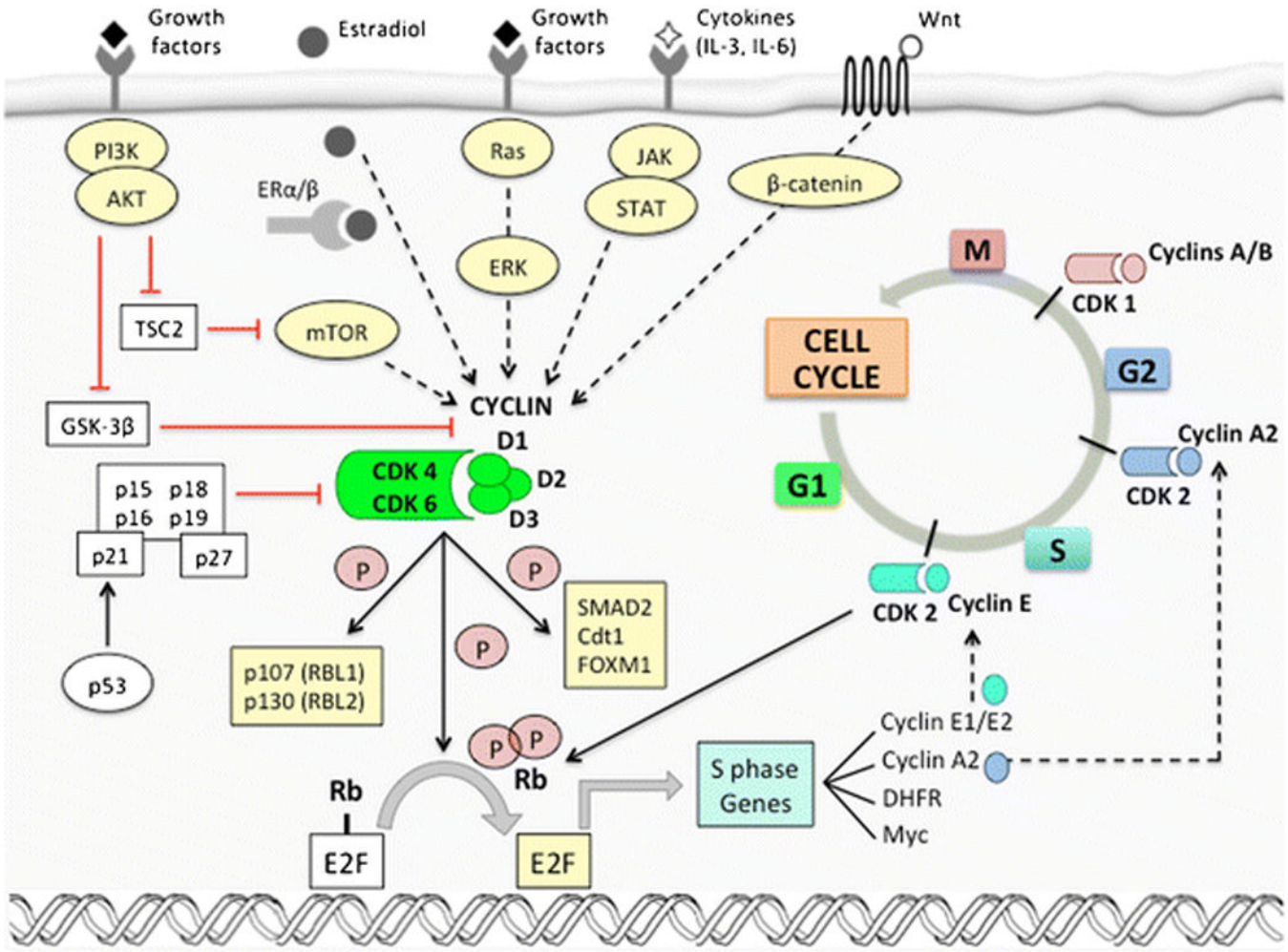

Figure 1.

The role of cyclins/cyclin-dependent kinases (CDK) in cell-cycle progression and the crosstalk with oncogenic signaling pathways.

Table 1

Results of phase II/III clinical trials with CDK4/6 inhibitors in advanced breast cancer

	PALBOCICLIB			RIBOCICLIB	ABEMACICLIB
	PALOMA-1/TRIO-18	**PALOMA-2**	**PALOMA-3**	**MONALEESA-2**	**MONARCH-1**
Study arms	Palbociclib + Letrozole vs. Letrozole	Palbociclib + Letrozole vs. Placebo + Letrozole	Palbociclib + Fulvestrant vs. Placebo + Fulvestrant (+/− Goserelin)	Ribociclib + Letrozole vs. Placebo + Letrozole	Abemaciclib
Trial design	Phase II Open-label	Phase III Double-blind	Phase III Double-blind	Phase III Double-blind	Phase II Single-arm
Menopause status inclusion criteria	Postmenopausal	Postmenopausal	All	Postmenopausal	All
Prior ET allowed for advanced BC	No	No	Yes	No	Yes
Pts, n	165	666	521	668	132
Dose CDK4/6 Inhibitor	125mg QD 3w on/1w off	125mg QD 3w on/1w off	125mg QD 3w on/1w off	600mg QD 3w on/1w off	200mg BID continuously
Median PFS (experimental arm vs. control arm), mo.	20.2 vs. 10.2	24.8 vs. 14.5	9.5 vs. 4.6	NR vs. 14.7	6.0
HR PFS (95% CI)	0.49 (0.32–0.75)	0.58 (0.46–0.72)	0.46 (0.36–0.59)	0.56 (0.43–0.72)	NA
Median OS (experimental arm vs. control arm), mo.	37.5 vs. 33.3	NA	NA	NA	17.7
HR OS (95% CI)	0.81 (0.49–1.35)	NA	NA	NA	NA
ORR (ITT; measurable disease), %	43 vs. 33; 55 vs. 39	42 vs. 35; 55 vs. 44	19 vs. 9; 25 vs. 11	41 vs. 28; 53 vs. 37	20; NA
CBR (ITT), %	81 vs. 58	85 vs. 70	67 vs. 40	80 vs. 73	42
Any grade 3–4 AE	76 vs. 21	76 vs. 24	69 vs. 18	81 vs. 33	NA
Grade 3–4 neutropenia, %	54 vs. 1	67 vs. 1	65 vs. 1	59 vs. 1	27
Grade 3–4 diarrhea, %	4 vs. 0	1 vs. 1	0 vs. 0	1 vs. 1	20

ET: endocrine therapy; BC: breast cancer; w: weeks; mo: months; pts: patients; n: number; NA: not available/not applicable; NR: not reached; PFS: progression-free survival; OS: overall survival; HR: hazard ratio; ORR: overall response rate; CBR: clinical benefit rate; ITT: intention-to-treat; AE: adverse event