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Cyclin-dependent kinase pathways as targets for women's cancer treatment

Gottfried E. Konecny

Purpose of review

In this article, we not only review the preclinical and clinical studies of cyclin-dependent kinase (CDK) 4/6 inhibitors in breast cancer, liposarcoma, mantle cell lymphoma, melanoma and germ cell tumors, but also examine promising preclinical data in glioblastoma, renal and ovarian cancer models that may provide directions for future development.

Recent findings

Targeting CDKs has been the focus of considerable basic science and clinical research. The CDK 4/6 inhibitors are a novel class of therapeutics that target the CDK 4/6 kinases that promote transition through the cell cycle. Currently, palbociclib (PD0332991, Pfizer), abemaciclib (LY2835219, Lilly) and ribociclib (LEE011, Novartis) are being investigated in clinical trials. These oral agents offer the hope of clinical efficacy in many tumor types, and have been associated with minimal toxicity. Amplification/overexpression of cyclin D, loss of *CDKN2A* (p16) and amplification/overexpression of *CDK4* are proposed biomarkers of improved response to CDK4/6 inhibition.

Summary

Palbociclib, abemaciclib and ribociclib have demonstrated very promising clinical activity in breast cancer, liposarcoma, mantle cell lymphoma and melanoma. Moreover, CDK4/6 inhibitors have shown promising preclinical activity in glioblastoma, renal and ovarian cancer models that may provide directions for their future clinical development. Further preclinical and clinical research is needed to better understand mechanisms of resistance and develop rational combination therapies with other targeted agents.

Keywords

abemaciclib (LY2835219) and ribociclib (LEE011), cyclin-dependent kinase 4 and 6 inhibition, palbociclib (PD0332991)

BACKGROUND

Cell cycle dysregulation is a common molecular finding in cancer and the cyclin-dependent kinases (CDKs) represent attractive targets in this pathway [1]. Under normal circumstances, the cell cycle functions as a tightly regulated process consisting of several distinct phases. Progression through the G1-S phase requires phosphorylation of the retinoblastoma (Rb) protein by CDK4 [2] or the highly homologous enzyme CDK6 [3] in complex with their activating subunits, the D-type cyclins, D1, D2 or D3 [4]. During the G1 cell cycle phase, at the restriction point, the cell has two options, to continue cycling or to enter the nondividing G0 state. As the cell passes through the restriction point, cyclin E complexes with CDK2 and hyperphosphorylates the Rb protein [5]. Hyperphosphorylation of the Rb protein diminishes its ability to repress gene transcription through the E2F family of

transcription factors and consequently allows synthesis of several genes that encode proteins, which are necessary for DNA replication [6]. Assembly of active cyclin D-CDK4/6 complexes is negatively regulated by INK4 protein family members p16, p15, p18 and p19 and by a second group of proteins including p21 and p27 [7]. As cells enter into S phase, cyclin A replaces cyclin E as the partner of CDK2 thereby enabling S phase to progress [8]. Later, in S phase cyclin A switches partners and associates instead with CDK1. As the cell moves

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KEY POINTS

- Cell cycle dysregulation is a common molecular finding in cancer and the CDKs represent attractive targets in this pathway.
- Amplification/overexpression of cyclin D, loss of *CDKN2A* (p16) and amplification/overexpression of *CDK4* are proposed biomarkers of improved response to CDK4/6 inhibition.
- Palbociclib, abemaciclib and ribociclib have demonstrated very promising clinical activity in breast cancer, liposarcoma, mantle cell lymphoma and melanoma.
- CDK4/6 inhibitors have shown promising preclinical activity in glioblastoma, renal and ovarian cancer models that may provide directions for their future clinical development.

further into the G2 phase, cyclin A is replaced by cyclin B and the cyclin B/CDK1 complex triggers mitosis [8].

TARGETING CDK4/6

Activation of the cyclin D-CDK4/6-Rb pathway can occur through several mechanisms. Amplification/overexpression of cyclin D1 and loss of *CDKN2A* (p16) has been described in breast cancer [9]. Amplification of *CDK4* is seen with high prevalence in well-differentiated and dedifferentiated liposarcomas [10]. In mantle cell lymphoma, the t(11;14) translocation places cyclin D under the control of the immunoglobulin gene promoter which leads to aberrant expression of cyclin D which is not expressed in normal B cells [11,12]. A high rate of homozygous deletion of *CDKN2A* has been described in melanoma [13]. Reduced expression of *CDKN2A* has been described in ovarian cancer [14] and is thought to occur through promoter methylation [15]. However, homozygous deletion of the *CDKN2A* gene and mutations have also been described in ovarian cancer [16,17]. Importantly, however, certain cancers are likely to be intrinsically resistant to CDK4/6 inhibition. For example, tumors that lack a functional Rb protein are likely not able to respond to CDK4/6 inhibition because the anti-tumor effect of CDK4/6 inhibition depends on a functional downstream Rb protein. Loss of Rb expression occurs in approximately 20% of breast cancers and is more commonly seen in triple negative breast cancer [18]. This group will also include cancers with functional inactivation of the Rb protein such as squamous cell cancer of the oral cavity or cervix and genital tract where the E7

oncogene of the human papillomavirus inactivates the Rb protein [19]. Nevertheless, the frequent alterations of the cyclin D-CDK4/6-Rb pathway in most cancers have led to ongoing efforts to block the pathway pharmacologically in a large number of different tumor types.

PALBOCICLIB

Palbociclib (PD0332991, Pfizer) is an orally active, potent and highly selective inhibitor of CDK4/6 kinases with the ability to block Rb phosphorylation in the low nanomolar range [20]. In a phase I dose escalation study, Flaherty *et al.* [21] treated 41 patients with Rb-positive advanced cancers with once-daily palbociclib (75, 125 and 150 mg) for 21 out of 28 days in repeated cycles. The maximum tolerated dose was 125 mg once daily, with neutropenia being the dose-limiting toxicity. Twenty-seven percent of patients had stable disease after a minimum of four cycles [21]. Finn *et al.* [22^{***}] have conducted the most comprehensive preclinical evaluation of palbociclib in breast cancer cell lines to date. Cell lines representing luminal estrogen receptor-positive (ER+) subtype (including those that are HER2 amplified) were most sensitive to growth inhibition by palbociclib whereas nonluminal/basal subtypes were the most resistant. Furthermore, palbociclib was synergistic with tamoxifen and trastuzumab in ER+ and HER2-amplified cell lines, respectively, and palbociclib enhanced sensitivity to tamoxifen in cell lines with conditioned resistance to ER blockade [22^{***}]. In February 2015, the FDA granted accelerated approval to palbociclib, for use in combination with letrozole for the treatment of postmenopausal women with ER+, HER2-negative advanced breast cancer as initial endocrine-based therapy for their metastatic disease. The approval is based on the randomized, multicenter, open-label phase II trial (PALOMA-1) in 165 patients who were randomly allocated to receive palbociclib (125 mg orally daily for 21 consecutive days, followed by 7 days off treatment) and letrozole (2.5 mg daily continuously throughout the 28-day cycle) or letrozole alone [23^{***}]. The trial was divided into two cohorts: cohort 1 enrolled 66 biomarker-unselected patients and cohort 2 enrolled 99 biomarker-positive (*CCND1* amplification and/or loss of *CDKN2A*). An improvement in progression-free survival (PFS) was observed in patients receiving palbociclib and letrozole compared with letrozole alone [hazard ratio (HR), 0.488 (95% CI: 0.319, 0.748); *P* < 0.001]. The estimated median PFS durations were 20.2 and 10.2 months in the palbociclib and letrozole and letrozole alone arm, respectively. Overall response rate in patients with

measurable disease was higher in the palbociclib and letrozole arm compared with the letrozole alone arm [55.4% (95% CI: 42.7, 67.7) versus 39.4% (95% CI: 27.6, 52.2)]. At the time of final analysis of PFS, overall survival (OS) data were not mature with 37% of events in favor of palbociclib and letrozole [HR, 0.813 (95% CI: 0.492, 1.345)]. Demonstrating an OS benefit in metastatic ER+ breast cancer trials is challenging, and has been a complex topic in the development of agents for this category of breast cancer. As for PALOMA-1, this was a randomized phase 2 trial that was not statistically designed to be able to determine whether an OS benefit is present with this drug. The ability to define whether an OS benefit is present typically requires a large trial with extended follow-up. Therefore, it is appropriate to wait for results from PALOMA-2, which is the larger randomized phase 3 study, for a readout on OS as study endpoint. Of note, patients in the palbociclib and letrozole arm had a longer median PFS compared with patients in the letrozole arm, regardless of biomarker status. In fact, the PFS improvement was more pronounced in the biomarker-unselected population. The biomarker analysis did not identify a population among these patients with ER+, HER2-negative advanced breast cancer that derived and improved benefit [23¹¹]. The most common adverse reaction was neutropenia (48% grade 3 and 6% grade 4). No case of febrile neutropenia was reported in PALOMA-1. The majority of grade 3 neutropenia was managed by dose reductions and or delays and did not require discontinuation of study drug or addition of growth factors [23¹¹].

Palbociclib has also been studied as a single agent in patients with endocrine-resistant advanced breast cancer [24]. Among 37 patients with metastatic breast cancer and positive Rb protein expression single-agent therapy with palbociclib led to two partial responses, five stable diseases more than 6 months for an overall clinical benefit rate of 19% [24]. Median PFS was 3.7 months. This study underscored that single-agent palbociclib was well tolerated and demonstrated activity in patients with endocrine-resistant ER+ breast cancer [24].

The PALOMA-3 study assessed palbociclib in combination with fulvestrant in patients with ER+, HER2-negative breast cancer that had relapsed or progressed during prior endocrine therapy. In this phase 3 study, 521 patients were randomly assigned in a 2:1 fashion to receive palbociclib and fulvestrant or placebo and fulvestrant [25¹²]. Premenopausal and perimenopausal women also received goserelin. The median PFS was 9.2 months with palbociclib and fulvestrant and 3.8 months with placebo and fulvestrant [HR, 0.42 (95% CI: 0.32, 0.56; $P < 0.001$)]. The most common grade 3 or 4

adverse event in the palbociclib fulvestrant group was neutropenia (62%). Again the majority of grade 3/4 neutropenia was managed by dose reductions and or delays and did not require discontinuation of study drug or the addition of growth factors. This double blind phase 3 randomized study confirmed that adding palbociclib to hormonal therapy results in substantially longer PFS than hormonal therapy alone in patients with advanced ER+, HER2-negative breast cancer that had progressed during prior endocrine therapy, irrespective of menopausal status. The adverse events were consistent with previously reported data. Most importantly, quality of life and the rate of treatment discontinuation because of adverse events were similar to that observed with the placebo [25¹²].

Palbociclib is currently also being evaluated for the treatment of primary breast cancer. In the adjuvant setting, the phase 3 PENELOPE-B study (NCT01864746) is accruing patients who have residual disease in the breast or lymph nodes after neoadjuvant chemotherapy and surgery. Approximately 800 patients with ER+, HER2-negative disease will be randomly assigned to receive either standard adjuvant endocrine therapy or endocrine therapy with palbociclib. In the preoperative setting, there are a number of additional trials being conducted in patients who have been diagnosed with ER+ breast cancer. These trials are looking at combinations of palbociclib with different endocrine therapies before surgery. Additional studies are being planned to investigate palbociclib in patients with advanced HER2-positive disease.

Palbociclib has also been studied in patients with well-differentiated and dedifferentiated liposarcomas [26]. Amplification of CDK4 is a characteristic feature of these tumors [27]. The 12-week PFS rate was reported to be 66% among 30 patients treated with single-agent palbociclib and one partial response was observed [26]. These results exceed clinical outcomes seen with conventional treatment regimens. All patients had received at least one prior regimen of systemic therapy and some had received up to five prior regimens. As expected, amplification of CDK4 was detected in more than 90% of samples which is consistent with prior published series [27].

Mantle cell lymphoma is characterized by the oncogenic *t(11;14)* translocation that results in aberrant expression of cyclin D [11,12]. In addition, CDK4 activity is further enhanced by concomitant homozygous deletion of *CDKN2A* which encodes the p16 protein [28]. Alternatively, p16 expression may also be downregulated in mantle cell lymphoma as a result of BMI-1 amplification, which causes overexpression of the

transcriptional repressor of the *CDKN2A* locus [29]. A pharmacodynamic study of palbociclib was conducted in 17 patients with relapsed mantle cell lymphoma using fluoro-D-glucose-PET to study tumor metabolism and proliferation. Five patients achieved PFS time longer than 1 year, with one complete and two partial responses (objective response rate 18%) [30].

A phase 2 study has been conducted in patients with treatment refractory germ cell tumors (GCTs), including mostly unresectable teratomas and teratomas with malignant transformations [31]. Upregulation of CDK4 and cyclin D2 has been described to be a central event in GCT tumorigenesis [32]. Moreover, cyclin D1 has been shown to be overexpressed in cisplatin-resistant GCT [33]. Among the 30 patients accrued to the study, all patients were diagnosed with metastatic nonseminomatous GCT and had received standard first-line cisplatin-based chemotherapy. The primary tumor site was testis in 20 patients, ovary in four patients and extragonadal in six patients. Eight of the 29 evaluable patients achieved 24-week PFS. The estimated 24-week PFS rate was 28%. This exceeded the proposed promising 24-week PFS rate of 15% that was specified in the study protocol [31].

Preclinical studies in glioblastoma, melanoma and renal or ovarian cancer models may provide directions for the future development of CDK4/6 inhibitors in other malignancies.

A preclinical study using 20 glioblastoma xenograft models reported that palbociclib was sensitive with cell lines which have a loss of p16, or phosphorylation of Rb [34]. Other preclinical studies using 25 renal cell carcinoma cell lines [35] or 40 ovarian cancer cell lines showed that the loss of p16 was significantly associated with high sensitivity [17]. Deregulation of the CDK4/6 signaling pathway is a common molecular finding in ovarian cancer [14]. When studying the effects of palbociclib in a large panel of ovarian cancer cell lines, representing the heterogeneity of ovarian cancer, sensitivity was most pronounced in ovarian cancer cell lines with low *CDKN2A* (p16) expression [17]. The results of these preclinical experiments also suggested that the increased expression of cyclin E, myc or aurora kinase A, which are often seen in ovarian cancer, may confer resistance to CDK4/6 inhibition in ovarian cancer cells (Fig. 1) [17]. Palbociclib is currently being evaluated as a single agent in recurrent ovarian cancer and correlative biomarker studies will help to better understand which patients with recurrent ovarian cancer are most likely to benefit from CDK4/6 inhibition (NCT01536743).

Although CDK 4/6 inhibitors appear to have a synergistic activity with antiestrogens, there are

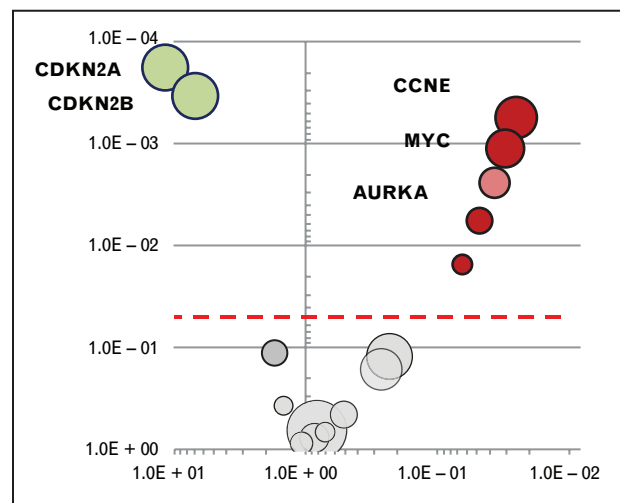


FIGURE 1. Volcano plot depicting preclinical activity of palbociclib across a panel of 40 ovarian cancer cell lines and gene expression of biomarkers associated with preclinical response. When studying the effects of palbociclib in this large panel of ovarian cancer cell lines [17], representing the heterogeneity of ovarian cancer, sensitivity was most pronounced in ovarian cancer cell lines with low *CDKN2A* (p16) and *CDKN2B* (p15) expression. The results of these preclinical experiments also suggested that the increased expression of *CCNE* (cyclin E), *MYC* (*myc*) or *AURKA* (aurora kinase A), which are often seen in ovarian cancer, may confer resistance to CDK4/6 inhibition in ovarian cancer cells.

limited preclinical data on the combination of CDK4/6 inhibitors with conventional chemotherapy. One study evaluated palbociclib and carboplatin in breast cancer xenograft models and demonstrated that the combination was associated with less antitumor activity than either carboplatin or palbociclib alone. On the basis of these data, the authors concluded that CDK 4/6 inhibitors should not be used in conjunction with carboplatin in tumors that are dependent on CDK4/6 [36].

ABEMACICLIB

Abemaciclib (LY2835219) is another CDK4/6 inhibitor in clinical development. Abemaciclib demonstrates activity in human xenograft tumor models [37]. Phase 1 trials have been completed with abemaciclib, and the agent is now in phase 2 and 3 studies of patients with ER+, HER2-negative breast cancer. In a phase 1 study with expansion cohorts, safety, pharmacokinetics and antitumor activity of abemaciclib were assessed in five different tumor types: glioblastoma; melanoma; and cancers of the lung, colon and breast [38]. In the expansion cohorts, abemaciclib was administered

continuously at 150–200 mg orally every 12 h on days 1–28 of a 28-day cycle. In the metastatic breast cancer cohort, 47 patients with a median of 7 prior systemic regimens received therapy with abemaciclib. Across all metastatic breast cancer patients, nine achieved a best overall response of confirmed partial response, 24 achieved stable disease, 11 had progressive disease and three were not evaluable for response. Among the 36 ER+ patients, there were nine confirmed partial responses for an objective response rate of 25%. Abemaciclib has some distinct features that set it apart from other CDK4/6 inhibitors. The drug has been shown to cross the blood–brain barrier [39]. This characteristic may be relevant in the treatment of patients with brain metastases, and suggests a rationale to study the activity of abemaciclib in this patient population. The principle adverse events were diarrhea, fatigue, nausea and neutropenia [38]. Possible differences in the adverse events that have been reported for each of the different CDK4/6 inhibitors may in part be related to known differences in the selectivity of these small molecule inhibitors for other CDKs such as CDK1 and will require further study to better understand possible differences in their toxicity profiles. Abemaciclib was also studied in combination with endocrine or HER2-targeted therapies for metastatic breast cancer. Patients in six cohorts received abemaciclib 150–200 mg every 12 h with letrozole 2.5 mg/day, anastrozole 1 mg/day, tamoxifen 20 mg/day, exemestane 25 mg/day, exemestane 25 mg/day + everolimus 5 mg/day or trastuzumab 6–8 mg/kg every 21 days. The disease control rate (PR + SD) was 67% for the nonsteroidal aromatase inhibitors (36 patients) with two confirmed PRs and 75% for tamoxifen (16 patients) [40]. Currently, a second-line study called MONARCH 1 is evaluating abemaciclib monotherapy for patients whose disease has progressed despite previous chemotherapy (NCT02102490). MONARCH 2 is looking at patients who are receiving fulvestrant with or without abemaciclib (NCT02107703), and MONARCH 3 is looking at abemaciclib as first-line treatment for women taking an aromatase inhibitor (NCT02246621). NEOMONARCH is a phase 2 neoadjuvant trial comparing the biological effects of 2 weeks of abemaciclib in combination with anastrozole with those of abemaciclib monotherapy and anastrozole monotherapy and evaluating the clinical activity and safety of subsequent therapy with abemaciclib either as single agent or in combination with anastrozole in postmenopausal women with hormone receptor-positive, HER2-negative breast cancer (NCT02441946).

RIBOCICLIB

A third selective inhibitor of CDK4/6 is ribociclib (LEE011, Novartis) which is also an orally bioavailable small molecule that inhibits CDK4/6 at nanomolar concentrations [41]. Ribociclib has demonstrated antitumor activity in several models, including melanoma with BRAF or NRAS mutations, and in breast cancer. Both intermittent and continuous doses were evaluated in a phase 1 trial. Patients with Rb-positive advanced solid tumors and lymphomas were treated with escalating doses of ribociclib for 3 weeks out of every 4 weeks or on a continuous schedule. The recommended phase 2 study dose was 600 mg/day for 3 weeks out of every 4 weeks. The most common adverse events (all grades) were neutropenia (40%), nausea (35%) and fatigue (27%). Grade 3/4 adverse events included neutropenia (19%). Among 70 evaluable patients, two had confirmed PRs (one patient with *PIK3CA*-mut, *CCND1*-amp, ER+ breast cancer; one patient with *BRAF*/*NRAS*-wild type, *CCND1*-amplified melanoma). Stable disease for at least four and six cycles was seen in 26 and 14% of the patients, respectively [42]. Ribociclib has also been combined with the MEK inhibitor binimetinib in a single arm phase 1b study in NRAS mutant malignant melanoma and has shown very promising preliminary clinical activity with partial responses seen in six of 14 (43%) patients [43]. Development of ribociclib is continuing in trials in patients with metastatic breast cancer. A phase 3 trial is under way with a similar design as PALOMA 2. In MONALEESA-2, 500 patients with previously untreated advanced hormone receptor-positive/HER2-negative breast cancer will be randomized to letrozole 2.5 mg daily with either ribociclib 600 mg daily for three out of every 4 weeks or placebo (NCT01958021). MONALEESA-3 is a randomized double-blind, placebo-controlled study of ribociclib in combination with fulvestrant for the treatment of 660 postmenopausal women with hormone receptor-positive, HER2-negative, advanced breast cancer who have received no or only one line of prior endocrine treatment (NCT02422615). The MONALEESA-7 study will investigate whether ribociclib given in combination with goserelin and tamoxifen or with goserelin and an aromatase inhibitor is effective at treating premenopausal women with advanced hormone receptor-positive/HER2-negative breast cancer (NCT02278120).

Preclinical studies have suggested that inhibitors of both CDK4/6 and phosphoinositide 3-kinase (PI3K) may have a synergistic effect in the treatment of tumors with *PIK3CA* mutations [44]. This observation, in addition to the known synergy between CDK4/6 inhibitors and endocrine

therapy, supports the exploration of ‘triplet’ therapy for metastatic ER+ breast cancer. A clinical trial evaluating the combination of ribociclib, the PI3K inhibitor BYL-719 and endocrine therapy is ongoing (NCT01872260). Moreover, a high-throughput RNAi kinome screen targeting 720 kinases was recently performed to identify potentially targetable molecules whose inhibition in combination with the CDK4/6 inhibitor ribociclib induced synthetic lethality in ER+ breast cancer cells. 3-phosphoinositide-dependent protein kinase 1 (PDK1) was identified as the top hit whose downregulation sensitized breast cancer cells to ribociclib. Pharmacological inhibition of PDK1 with the ATP-competitive, small molecule inhibitor GSK2334470 synergistically inhibited proliferation of breast cancer cells. Moreover, ribociclib-resistant breast cancer cells displayed increased PDK1 protein expression compared with parental cells. Inhibition of PDK1 with GSK2334470 resensitized the ribociclib-resistant cells to the CDK4/6 inhibitor. These studies suggest that PDK1 inhibition can overcome resistance to the CDK4/6 inhibitor ribociclib and may offer a rationale for further translational and clinical investigation of combinations of CDK4/6 and PDK1 inhibitors [45].

CONCLUSION

The development of selective CDK4/6 inhibitors has changed the perception of CDKs as therapeutic targets in cancer. Palbociclib, abemaciclib and ribociclib have demonstrated very promising clinical activity in breast cancer, liposarcoma, mantle cell lymphoma and melanoma. Moreover, CDK4/6 inhibitors have shown promising preclinical activity in glioblastoma, renal and ovarian cancer models that may provide directions for their future clinical development. Taken together, the data summarized in this review support further clinical evaluation of palbociclib, abemaciclib and ribociclib as a single agent or in combination with antihormonal or other targeted agents in women’s cancers. Moreover, the assessment of functionally implicated response predictors in these clinical trials may help to identify the patient subgroup most likely to benefit from treatment with CDK4/6 inhibitors.

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Conflicts of interest

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