Introduction

The cyclin D-cyclin-dependent kinase (CDK) 4/6-inhibitor of CDK4 (INK4)-retinoblastoma (Rb) pathway regulates cellular proliferation by controlling the G1–S checkpoint. Dysregulation of the cyclin D-CDK4/6-INK4-Rb pathway is frequently observed in cancer and contributes to cell cycle progression and continued growth [1]. CDK4/6 mediates the transition from G1 to S phase by associating with D-type cyclins and regulating the phosphorylation state of Rb (Fig. 1A). Unphosphorylated Rb binds and represses the function of E2 family (E2F) transcription factors; upon phosphorylation, Rb dissociates from E2F transcription factors, freeing them to be able to participate in DNA replication and cell division. Increased cyclin D-CDK4/6 activity, which promotes phosphorylation of Rb, can occur through several mechanisms, including overexpression of D-type cyclins, mutation or amplification of CDK4/6, or loss of cyclin D-CDK4/6 negative regulators such as p16INK4A [1–3], and ultimately leads to cancer cell growth. Thus, the development of selective CDK4/6 inhibitors offers a novel therapeutic approach for patients with advanced cancer.

This review will discuss how the cyclin D-CDK4/6-INK4-Rb pathway becomes dysregulated, the resulting effects of pathway activation, and the rationale for inhibiting CDK4/6 in cancer. It will also provide an overview of the preclinical and clinical data for key CDK4/6 inhibitors currently under investigation, and will examine combination approaches that may be exploited in various cancer types.

Overview of the cyclin D-CDK4/6-INK4-Rb pathway in cancer

The cell cycle is broadly divided into four sequential phases: G1 (pre-DNA synthesis), S (DNA synthesis), G2 (pre-division), and M (cell division). The key regulator of the G1–S transition is the cyclin D-CDK4/6-INK4-Rb pathway (Fig. 1A) [1]. CDK4 and CDK6 share 71% amino acid identity and have largely overlapping functions [4]. Both kinases are expressed in most cell types—albeit to different extents—and both can partner with all three D-type cyclins (D1, D2, and D3) [4]. Upon activation by mitogenic signaling pathways, D-type cyclins associate with CDK4 or CDK6. These active cyclin D-CDK4/6 complexes phosphorylate Rb, and thereby...
promote dissociation of the transcriptionally repressive Rb-E2F complex. The released E2F transcription factors are then free to activate genes required for entry into S phase and DNA replication [1,5].

CDK4/6 activity is regulated by the INK4 family of proteins (p16INK4A, p15INK4B, p18INK4C, and p19INK4D) and by the Cip and Kip family (particularly p21CIP1 and p27KIP1) [5]. The INK4 proteins inhibit cyclin D-CDK4/6 activity by directly binding to the CDK [5]. Of the four INK4 proteins, p16INK4A appears to be particularly important for tumor suppression [1]. p21CIP1 and p27KIP1 have context-dependent positive and negative effects on CDK4/6 activity; both can stabilize cyclin D-CDK4/6 complexes and both possess CDK4/6-inhibitory activity under certain conditions, creating a complex system of regulation [6].

While loss of Rb is responsible for G1-S transition in some cancer types, the vast majority of cancers retain wild-type Rb [1]. These Rb-positive (Rb+) cancers can instead be driven by overexpression or activation of cyclin D-CDK4/6, loss of cyclin D-CDK4/6 negative regulators, or by oncogenic signaling pathways that promote cyclin D-CDK4/6 activity, and are likely to be dependent on CDK4/6 activity for cell proliferation. Key oncogenic signaling pathways that promote cyclin D-CDK4/6 activity include phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of
or CDK6, or deletion of the locus encoding p16 INK4A and loss of p16INK4A in 49% [9,10].

CDK4 at R24 prevents p16INK4A binding, resulting in enhanced pathway activation. For example, mutation of liposarcoma and neuroblastoma [15–18].

Mechanisms of cyclin D-CDK4/6-INK4-Rb pathway activation

Amplification of the genes encoding cyclin D1 (CCND1), CDK4, or CDK6, or deletion of the locus encoding p16INK4A (cyclin-dependent kinase inhibitor 2A [CDKN2A]) are major mechanisms by which the cyclin D-CDK4/6-INK4-Rb pathway can be activated. For example, the ProFiLER study (NCT01774409) characterized genomic alterations in advanced cancers (N = 105; breast, head and neck, and lung cancers accounted for >50% of cases) and identified amplifications in CCND1 (17% of cases), CDK4 (4%), and CDK6 (3%), as well as homozygous deletion of CDKN2A [21%] [8]. Other studies in breast cancer have reported CDK4 amplification in 16% of cases, CDK6 amplification in 17%, and loss of p16INK4A in 49% [9,10]. CCND1 amplification is seen in up to 35% of breast cancer cases, while the cyclin D protein is overexpressed in over 50% [9,11]. Amplification of CCND1 is also frequently observed in head and neck cancer (26–39% of cases), non-small cell lung cancer (NSCLC; 5–30%), endometrial carcinoma (26%), and pancreatic cancer (25%) [11]. Similarly, almost 50% of prostate cancers overexpress CDK6 [12], and overexpression or copy number variation of at least one cyclin D-CDK4/6-INK4-Rb pathway component occurs in most cases of melanoma (approximately 75%) [13], head and neck squamous cell carcinoma (loss of p16INK4A in approximately 80%) [14], and almost all cases of liposarcoma and neuroblastoma [15–18].

Similarly, specific mutations or translocations in the genes encoding cyclin D-CDK4/6-INK4-Rb pathway components can also result in aberrant pathway activation. For example, mutation of CDK4 at R24 prevents p16INK4A binding, resulting in enhanced kinase activity [5]. CDK4 R24 mutations were first characterized in familial melanoma [19], and were identified in 2.5% of melanoma cases [13], head and neck squamous cell carcinoma (loss of p16INK4A in approximately 80%) [14], and almost all cases of liposarcoma and neuroblastoma [15–18].

Inhibiting CDK4/6 in cancer

Due to the importance of CDK4/6 activity in regulating cell proliferation and the mechanisms by which this pathway is known to be activated in cancer, selective inhibition of CDK4/6 inhibitors has emerged as an attractive therapeutic strategy [32]. A major theoretical concern is that CDKs play a critical role in the proliferation of normal cells as well as cancer cells, potentially creating a narrow therapeutic window where toxicity would limit the ability to attain clinically effective exposure levels. To date, the most well-studied pan-CDK inhibitor is flavopiridol, which showed limited clinical benefit, partly due to its complex pharmacokinetics and high levels of off-target side effects [1,33]. It is possible that cancers with known aberrations in the cyclin D-CDK4/6-INK4-Rb pathway will be more sensitive to CDK4/6 inhibition than normal cells [32]. Furthermore, selective inhibitors spare CDK2 activity which allows normal cells to continue to function and proliferate. Highly selective CDK4/6 inhibitors may therefore have a wider therapeutic window and fewer off-target toxicities than pan-CDK inhibitors.

Currently there are three CDK4/6 inhibitors that are either approved or in late-stage development (Fig. 1B): palbociclib (PD-0332991; Pfizer), ribociclib (LEE011; Novartis), and abemaciclib (LY2835219; Lilly). All three compounds inhibit CDK4 and CDK6 with IC50 values <40 nM, although they exhibit varying IC50 values against other CDKs (Table 1); such differences in selectivity may influence their optimal dosing schedules and side effect profiles (see below). All three compounds have also demonstrated preclinical activity in a range of Rb+ tumor models (Table 1) [5,34–38]. Interestingly, cell lines with loss or mutation of CDKN2A, or loss of p16INK4A protein expression, are particularly sensitive to palbociclib [13,39–41], although other preclinical models suggest that CDK4/6 inhibition may still be an effective strategy in tumors lacking these alterations [35,42].

Although CDK4/6 inhibition reduces proliferation and leads to cell cycle arrest, cellular senescence or senescence-like activity are likely to be important mechanisms associated with clinical activity. Cellular senescence is the process by which a cell undergoes permanent, irreversible growth arrest in response to cellular stresses (e.g. unresolved DNA damage), rendering it resistant to mitogenic stimulation and oncogenic challenge [43]. While a variety of biomarkers for cellular senescence exist, there is currently a lack of consensus regarding the exact biomarkers or combination of biomarkers to accurately measure senescence, which can confound interpretation of data regarding cellular activity [43]. Nevertheless, palbociclib-induced, senescence-associated (SA) β-galactosidase activity was markedly higher in melanoma cells than in normal melanocytes, indicating that melanoma cells are particularly reliant on CDK4/6-mediated suppression of senescence or senescence-like activity [44]. One possible mechanism of CDK4/6 inhibitor-induced senescence is through downregulation of the forkhead box M1 (FOXM1) transcription factor [44]. Indeed, ribociclib-induced reduction in FOXM1 messenger RNA (mRNA) and protein levels in ribociclib-sensitive neuroblastoma cell lines was associated with induction of cellular senescence, as measured using SA β-galactosidase activity [45]. In contrast, ribociclib-resistant cell lines showed no reduction of FOXM1.
mRNA or protein following ribociclib treatment, and did not undergo senescence or senescence-like activity [45]. For abemaciclib, a pharmacokinetic/pharmacodynamic model, using for at least 10 cycles (of 37 evaluable patients) [47].


disease (SD) for at least 4 cycles and six patients (16%) having SD achieved stable disease (SD) for at least 4 cycles and six patients (16%) having SD for at least 10 cycles (of 37 evaluable patients) [47].

Palbociclib monotherapy has since been studied in a variety of tumor types. In a single-arm study, five of 17 patients with relapsed MCL remained progression-free for more than 1 year on palbociclib therapy, with one complete response (CR) and two partial responses (PRs) [48]. In a phase II trial of palbociclib in 28 patients with metastatic Rb+ breast cancer, two patients achieved PR [49]. In a separate phase II trial in 30 patients with Rb+ advanced well-differentiated liposarcoma (WDLS) or dedifferentiated liposarcoma (DDLs), palbociclib treatment resulted in a 12-week progression-free survival (PFS) rate of 66% [50]. Finally, in a phase II trial of palbociclib in 19 patients with previously treated, advanced NSCLC exhibiting Rb expression and CDKN2A inactivation (negative p16INK4A expression), the median PFS was 12.5 weeks, and five patients remained on study for at least 24 weeks [51].

**Monotherapy approaches with CDK4/6 inhibitors**

**Palbociclib**

The first-in-human phase I dose escalation study of palbociclib was conducted in 41 patients with advanced solid tumors [47], who were prescreened to confirm the presence of Rb expression (Rb+). The maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of single-agent palbociclib were declared to be 125 mg/day on a 21-of-28 day schedule (Table 1). Five patients (12%) experienced dose-limiting toxicities (DLTs), all of which were neutropenia. Beyond Cycle 1, the most common grade 3 adverse events were neutropenia (n = 5; 12%) and anemia (n = 3; 7%; Table 2). The most common all-grade non-hematologic adverse events after Cycle 1 included fatigue (n = 10; 24%), diarrhea (n = 6; 15%), nausea, dyspepsia, and arterialized (n = 5; 12% each). Pharmacokinetic exposures to palbociclib following a single dose were generally dose-proportional. At steady state (Day 21), palbociclib was absorbed with a median Tmax of approximately 5.5 h and half-life of approximately 26 h. With repeated dosing, the median accumulation ratio was 2.2. Pharmacodynamic decreases in neutrophil and platelet counts correlated with increasing palbociclib exposure. During the 7-day rest period in Cycle 1, both cell types recovered, with platelet levels exceeding baseline values; indicating this effect was fully reversible. Preliminary signs of clinical activity were observed, with ten patients (27%) achieving stable disease (SD) for at least 4 cycles and six patients (16%) having SD for at least 10 cycles (of 37 evaluable patients) [47].

**Ribociclib**

The initial phase I dose escalation study of single-agent ribociclib enrolled 128 patients with Rb+ advanced solid tumors and lymphomas (NCT01237236). The MTD and RP2D were declared as 900 and 600 mg/day, respectively, on a 21-of-28-day schedule (Table 1) [52]. DLTs included neutropenia (n = 3), thrombocytopenia (n = 2), mucositis, pulmonary embolism, hyponatremia, increased creatinine, and asymptomatic QTc prolongation (n = 1 each). The most common treatment-related adverse events were neutropenia (45%), leukopenia (44%), nausea (43%), and fatigue (42%) [52]. Grade 3/4 adverse events included neutropenia, leukopenia, and lymphopenia (Table 2). Asymptomatic QTc corrected with Fridericia’s formula (QTcF) prolongation (>450 ms) was seen at doses of greater than 600 mg/day (10% of patients at 600 mg/day and 27% of patients at higher doses) [52]. The half-life was approximately 36 h, and paired skin biopsies from 40 patients showed reductions of at least 50% from baseline in Ki67 and phosphorylated Rb, in 55% and 42% of the samples, respectively. Among 110 evaluable patients, three had confirmed PRs: one patient with head and neck acinar carcinoma and CDKN2A loss, one patient with PIK3CA-mutant, CCND1-amplified, estrogen receptor-positive (ER+) breast cancer, and one...
The first-in-human phase I trial of abemaciclib enrolled 75 patients with advanced solid tumors [54]. The dose escalation phase explored once-daily and twice-daily (every 12 h) dosing on a 21-of-28-day schedule. Although the MTD for once-daily dosing was not reached, the MTD for twice-daily dosing was declared as 200 mg. The DLT was grade 3 fatigue in one of six patients at 200 mg and two of three patients at 275 mg. The most common all-grade treatment-related adverse events included diarrhea (52%), nausea (32%), fatigue, vomiting (21% each), and neutropenia (19%). The most common grade 3 treatment-related adverse events were diarrhea, neutropenia (7% each), fatigue, and nausea (5% each). At 200 mg, mean $C_{\text{max}}$ at steady state was 291 ng/mL and AUC$_{0-24h}$ was 5798 ng·h/mL. Pharmacodynamic evidence of targeted CDK4/6 inhibition was observed, as shown by a decrease in Rb phosphorylation in the skin. One patient with CDKN2A $^+$, KRAS-wild-type, CDK4/6 inhibitors was confirmed PR, and prolonged SD or minor radiographic decreases were seen in patients with ovarian cancer, NSCLC, and breast cancer [54]. One of the expansion cohorts from this study enrolled 57 patients with NSCLC [55]. There were 2 PRs, and 12 patients (of which 8 had KRAS-mutant disease) received treatment for >6 months. Another expansion cohort enrolled 47 patients with metastatic breast cancer [56]. Nine PRs were observed, all in patients with HR+ breast cancer (Table 2): the median PFS was 5.8 months, rising to 9.1 months for patients with HR+ breast cancer [56]. Based on these results, in October 2015 the Food and Drug Administration (FDA) granted abemaciclib a ‘Breakthrough Therapy’ designation with ‘Fast Track’ status for the treatment of advanced breast cancer [57].

### Table 2

Single-agent clinical trials of CDK4/6 inhibitors.

<table>
<thead>
<tr>
<th>Trial details</th>
<th>Grade 3/4 adverse events overall (≥10% of patients)</th>
<th>Clinical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abemaciclib</td>
<td>Neutropenia (20%), leukopenia (10%) in Cycle 1</td>
<td>27% SD for ≥4 cycles; 16% SD for ≥10 cycles (n = 37 evaluable)</td>
</tr>
<tr>
<td>Ribociclib</td>
<td>Neutropenia (36%), thrombocytopenia (24%), hypophosphatemia (12%)</td>
<td>6% CR, 12% PR, 41% SD (n = 16 evaluable)</td>
</tr>
<tr>
<td></td>
<td>Neutropenia (50%), thrombocytopenia (21%)</td>
<td>7% PR, median PFS 4.1 months for ER+/HER2−, 18.8 months for ER+/HER2+, 1.8 months for ER−/HER2− patients (n = 28 evaluable)</td>
</tr>
<tr>
<td></td>
<td>Neutropenia (50%), leukopenia (47%), thrombocytopenia (30%), lymphopenia (27%), anemia (17%)</td>
<td>3% PR, 12-week PFS 66%, median PFS 18 weeks (n = 29 evaluable)</td>
</tr>
</tbody>
</table>

CDK, cyclin-dependent kinase; CDK4/6, cyclin-dependent kinase inhibitor 2A/2B; CR, complete response; DCR, disease control rate; ER, estrogen receptor; ER+, ER-positive; ER−, ER negative; HER2, human epidermal growth factor receptor 2; HER2+, HER2-positive; HER2−, HER2 negative; HR+, hormone receptor-positive; INK4, inhibitor of CDK4; KRAS, K-Ras2 Kirsten rat sarcoma viral oncogene homolog; MCL, mantle cell lymphoma; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; PFS, progression-free survival; PK, pharmacokinetic; PR, partial response; Rb, retinoblastoma; Rb+, Rb-positive; RP2D, recommended phase II dose; SD, stable disease.

A second study investigated ribociclib in pediatric patients (aged 1–21 years) with MRT, neuroblastoma, and cyclin D-CDK4/6-INK4-Rb pathway-activated tumors. In the dose escalation portion of this phase I study, the MTD and recommended dose for expansion (RDE) were declared to be 470 mg/m²/day (adult equivalent dose ~800/600 mg/day) and 350 mg/m²/day (adult equivalent dose ~600 mg/day), respectively, on a 21-of-28-day schedule [56]. Ribociclib was well tolerated in the pediatric population, with a similar safety profile to that seen in adult patients [53].

Abemaciclib

The first-in-human phase I trial of abemaciclib enrolled 75 patients with advanced solid tumors [54]. The dose escalation phase explored once-daily and twice-daily (every 12 h) dosing on a continuous schedule. Although the MTD for once-daily dosing was not reached, the MTD for twice-daily dosing was declared as 200 mg. The DLT was grade 3 fatigue in one of six patients at 200 mg and two of three patients at 275 mg. The most common all-grade treatment-related adverse events included diarrhea (52%), nausea (32%), fatigue, vomiting (21% each), and neutropenia (19%). The most common grade 3 treatment-related adverse events were diarrhea, neutropenia (7% each), fatigue, and nausea (5% each). At 200 mg, mean $C_{\text{max}}$ at steady state was 291 ng/mL and AUC$_{0-24h}$ was 5798 ng·h/mL. Pharmacodynamic evidence of targeted CDK4/6 inhibition was observed, as shown by a decrease in Rb phosphorylation in the skin. One patient with CDKN2A $^+$, KRAS-wild-type, CDK4/6 inhibitors was confirmed PR, and prolonged SD or minor radiographic decreases were seen in patients with ovarian cancer, NSCLC, and breast cancer [54]. One of the expansion cohorts from this study enrolled 57 patients with NSCLC [55]. There were 2 PRs, and 12 patients (of which 8 had KRAS-mutant disease) received treatment for >6 months. Another expansion cohort enrolled 47 patients with metastatic breast cancer [56]. Nine PRs were observed, all in patients with HR+ breast cancer (Table 2): the median PFS was 5.8 months, rising to 9.1 months for patients with HR+ breast cancer [56]. Based on these results, in October 2015 the Food and Drug Administration (FDA) granted abemaciclib a ‘Breakthrough Therapy’ designation with ‘Fast Track’ status for the treatment of advanced breast cancer [57].

Combination approaches with CDK4/6 inhibitors

The greatest benefit of CDK4/6 inhibitors will likely lie in combination with other therapies, and a range of phase III trials are currently ongoing (Table 3).
Hormonal therapy

Hormonal therapy (or endocrine therapy) is the standard-of-care treatment for HR+ breast cancer. HR+ breast cancers express either or both of the estrogen receptor (ER) or progesterone receptor (PgR), and account for up to 70% of all breast cancers. Endocrine therapy blocks the hormone signaling that HR+ breast cancer cells rely on to grow and divide; however, up to 50% of HR+ breast cancers become resistant to endocrine therapy (acquired resistance) or demonstrate initial (de novo) resistance [7]. Endocrine therapy-resistant breast cancer cell lines and primary luminal B breast cancers, which are known to respond poorly to endocrine therapy, exhibit cyclin D-CDK4/6-INK4-Rb pathway activation and may be sensitive to CDK4/6 inhibition [58]. In addition, CCND1 amplification has been identified in up to 35% of breast cancers, while overexpression of the cyclin D protein is even more frequent [9,11,59,60]. Palbociclib has been shown to inhibit proliferation of endocrine therapy-resistant breast cancer cells in preclinical models [58]. Moreover, addition of ribociclib to letrozole or fulvestrant enhanced inhibition of tumor growth in ER+ xenograft models [61].

PALOMA-1/TRIO-18 was the first randomized, placebo-controlled phase II trial to test the addition of CDK4/6 inhibitor (palbociclib) to an aromatase inhibitor (letrozole) as initial treatment for postmenopausal women with ER+ human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer (N = 165). Combination of palbociclib with letrozole almost doubled PFS from 10.2 months to 20.2 months compared with letrozole alone (hazard ratio [HR] = 0.488, p = 0.0004) [62].

No biomarker was identified that predicted response to palbociclib; increased PFS was observed regardless of CCND1 amplification and loss of p16INK4A [62]. An interim overall survival (OS) analysis suggested a trend in favor of the palbociclib plus letrozole combination (37.5 vs 33.3 months; HR = 0.813, p = 0.042) [62]. Based on the results of PALOMA-1, the combination of palbociclib and letrozole was granted accelerated FDA approval in February 2015 for the first-line treatment of HR+ metastatic breast cancer [63]. Continued approval is contingent on the results of a larger phase III study (PALOMA-2; NCT01740427), identical in design to PALOMA-1, which has completed accrual and is in the follow-up phase [64].

Addition of palbociclib to endocrine therapy has also demonstrated clinical efficacy in the second-line setting. In the phase III PALOMA-3 study (NCT01942135) of palbociclib in combination with fulvestrant in women (any menopausal status) with HR+, HER2-negative advanced breast cancer after failure on endocrine therapy, palbociclib significantly increased PFS compared with placebo (median PFS 9.2 vs 3.8 months; HR = 0.42; 95% CI 0.32–0.56; p < 0.001) [65]. Palbociclib is additionally under investigation in the adjuvant and neoadjuvant settings. For example, a phase III trial of adjuvant palbociclib with standard endocrine therapy is ongoing in patients with HR+, HER2-negative breast cancer with residual disease after neoadjuvant chemotherapy and surgery (PENELOPE-B; NCT01864746). A phase II neoadjuvant study of palbociclib in combination with letrozole is also ongoing in ER+, HER2-negative breast cancer (PALLET; NCT02296801) [64].

Combination of ribociclib plus letrozole has been investigated in a phase Ib study for first-line treatment of postmenopausal...
patients with advanced ER+, HER2-negative breast cancer (NCT01872260) [64]; preliminary data suggest an acceptable safety profile and signs of clinical activity [66]. A phase III trial of ribociclib plus letrozole in this setting has now completed accrual of postmenopausal patients with advanced HR+, HER2-negative breast cancer (NCT01958021; MONALEESA-2) [64]. A companion study for premenopausal patients with HR+, HER2-negative advanced breast cancer (NCT02278120; MONALEESA-7) is investigating ribociclib plus letrozole or tamoxifen and is currently accruing patients [64]. The combination of ribociclib with fulvestrant is being investigated in the first- and second-line setting in the phase III MONALEESA-3 trial (NCT02422615), which is enrolling postmenopausal patients with advanced HR+, HER2-negative breast cancer [64].

The success of single-agent abemaciclib in HR+ metastatic breast cancer prompted expansion of the phase I study to evaluate abemaciclib (200 mg twice daily) in combination with the anti-estrogen fulvestrant (500 mg once monthly) in this patient population. In an interim analysis, 18 patients with a median of 4 prior systemic therapies had been treated; combination treatment had an acceptable safety profile and achieved a disease control rate of 72% [67]. Abemaciclib is also being investigated in combination with endocrine or HER2-targeted therapies in a phase Ib study in metastatic breast cancer [68]. Phase III studies that are underway in postmenopausal breast cancer include the MONARCH 2 study (NCT02107703) of abemaciclib plus fulvestrant in advanced HR+,HER2-negative breast cancer and the MONARCH 3 study (NCT02246621) of abemaciclib in combination with an NSAID (letrozole or anastrozole) in recurrent or metastatic HR+, HER2-negative breast cancer [64].

**PI3K/AKT/mTOR pathway inhibitors**

The PI3K/AKT/mTOR pathway is a key upstream regulator of cyclin D-CDK4/6 activity. In preclinical studies, palbociclib had an additive effect on cell death in pancreatic ductal adenocarcinoma (PDA) cell lines when combined with MEK or PI3K/mTOR inhibitors [69]. Additionally, combination of palbociclib with the mTOR complex 1 inhibitor everolimus synergistically inhibited the growth of NSCLC cell lines [70]. Single-agent ribociclib inhibited tumor growth in ER+ xenograft models, and this inhibition was further enhanced by the addition of letrozole or fulvestrant. The greatest tumor inhibition was observed with the triplet combination of ribociclib plus a PI3K inhibitor (buparlisib [BKM120] or alpelisib [BYL719]) and letrozole or fulvestrant [61].

Based on this preclinical work, there is significant interest in using CDK4/6 inhibitors in triplet combinations with endocrine therapy and an inhibitor of the PI3K/AKT/mTOR pathway. A randomized phase Ib study in ER+, HER2-negative advanced breast cancer (NCT01872260) is investigating the efficacy of first-line triplet therapy with ribociclib, letrozole, and alpelisib [64,71]. A phase Ib trial of combination ribociclib, everolimus, and exemestane in patients with ER+, HER2-negative metastatic breast cancer (NCT01857193) is also ongoing [64,72]. Similarly, a study of ribociclib and fulvestrant with or without buparlisib or alpelisib in patients with pretreated ER+, HER2-negative locally advanced or metastatic cancer is ongoing (NCT02508868) [64].

In a preclinical study, ribociclib also demonstrated synergistic activity with the PI3K inhibitors pictilisib (CDC-0941) or alpelisib in PI3K inhibitor-resistant, PIK3CA-mutant breast cancer cell lines, and produced tumor regression in PIK3CA-mutant breast cancer mouse models [73]. The phase II FERG1 study, reported at the San Antonio Breast Cancer Symposium in 2014, showed a modest, non-statistically significant benefit (6.6 vs 5.1 months, HR = 0.74; p = 0.0959) associated with the addition of pictilisib to fulvestrant in aromatase inhibitor-resistant, HR+ metastatic breast cancer [74]. Interestingly, although there was no increased benefit in PIK3CA-mutated tumors, there was an improved response in patients with PgR+ tumors (median PFS, 7.4 vs 3.7 months; p = 0.002) [74]. Further investigations will be necessary to evaluate the potential role of CDK4/6 and PI3 K pathway inhibitor combinations for treating HR+ breast cancer, and for determining biomarkers that are predictive of response.

**RAS/RAF/MEK/ERK pathway inhibitors**

Combination of CDK4/6 inhibitors with RAS/RAF/MEK/ERK pathway inhibitors is a promising therapeutic approach, particularly in patients with melanoma. The FDA has approved BRAF and MEK inhibitors both individually and in combination for patients with BRAF V600-mutant melanomas [75]. Despite high initial response rates, acquired resistance often develops faster than desired, limiting the durability of response with these targeted therapies. The addition of a CDK4/6 inhibitor has the potential to overcome some of these resistance mechanisms [76,77]. Melanoma cell lines and tumor models that are resistant to the BRAF inhibitor vemurafenib have reactivated MAPK signaling and upregulated cyclin D1, and are sensitive to abemaciclib treatment [76]. A phase Ib/II study combining ribociclib with the MEK inhibitor binimetinib (MEK162) is ongoing in patients with NRAS-mutant melanoma (n = 14; NCT01781572); one patient achieved a confirmed PR, and unconfirmed PRs were reported in a further five patients [77]. Although no pharmacokinetic interaction was observed, finding the right dose and schedule to maximally inhibit both pathways while minimizing toxicity remains important; treatment-related toxicities were common and included creatinine phosphokinase elevation, rash, edema, anemia, nausea, diarrhea, and fatigue. Triplet combinations of ribociclib with BRAF and MEK inhibitors are also currently being explored [64].

**Chemotherapy**

Preclinical studies show conflicting results regarding the benefit of combining CDK4/6 inhibitors with standard cytotoxic chemotherapy. Abemaciclib enhanced the antitumor effect of gemcitabine in a lung cancer xenograft model [37] and CDK4/6 inhibition sensitized neuroblastoma cells to doxorubicin-induced apoptosis [78]; in contrast, palbociclib reduced the cytotoxicity of antimicrotubule and platinum agents in preclinical models [32,69,79]. Interestingly, six PRs were recorded in a phase I trial of patients with pretreated, RB+ metastatic breast cancer who received a combination of palbociclib and paclitaxel (n = 15; NCT01320592) [80]. In this study, uncomplicated grade 3/4 neutropenia frequently led to dose reduction or dose interruption, and an expansion study is underway to investigate the effect of a 3-day (rather than 5-day) dosing schedule [80]. Thus, even if CDK4/6 inhibitors are found to improve the antitumor effects of chemotherapy, the ability to circumvent the overlapping toxicity of bone marrow suppression will remain a challenge in the clinic.

**Radiotherapy**

Emerging data suggest that inhibition of the cyclin D-CDK4/6-INK4-Rb pathway may sensitize cell lines to radiotherapy. A subpopulation of tumor cells, referred to as cancer stem cells, is typically resistant to radiotherapy [81]. These cells proliferate slowly, remain in a quiescent state for extended periods of time, and have the ability to reconstitute tumors [81]. Evidence points to a role for the cyclin D-CDK4/6-INK4-Rb pathway in
regulating quiescence and promoting radiotherapy resistance in cancer stem cells. Repression of CDK4 by the NFATc1 transcription factor appears to be involved in maintenance of quiescence in hair follicle stem cells [82]. In hematopoietic stem cells, CDK6 reportedly acts as a regulator of quiescence exit, with the absence of CDK6 protein resulting in a notable delay in quiescence exit [83]. In a study of a BCR-ABL<sup>Δ210+</sup>-induced leukemia cell line, CDK6-deficient stem cells tended to remain in the quiescent state compared with CDK6-proficient stem cells [84]. In addition, mice injected with CDK6-proficient BCR-ABL<sup>Δ210+</sup> bone marrow developed leukemia within 3 months, whereas mice injected with CDK6-deficient BCR-ABL<sup>Δ210+</sup> bone marrow generally failed to develop disease, further supporting a role for CDK6 in cancer stem cell activity [84]. In triple-negative breast cancer cell lines, Rb-deficient cancer stem cells exhibited increased response to radiotherapy compared with Rb-proficient cancer stem cells [85]. Radiotherapy-resistant cancer stem cells isolated from liver cancer and glioblastoma cell lines demonstrated upregulation of AKT and cyclin D-CDK4 signaling; inhibition of AKT, cyclin D, or CDK4 resulted in re-sensitization to radiotherapy in these cells [86]. In another study, radiotherapy-resistant cell lines knocked down for CDK4 demonstrated radiosensitivity, while cells knocked down for CDK2 were not re-sensitized [87]. This study also suggested that silencing CDK4/6 increases radiation-induced cell death but does not significantly alter cell cycle progression or interfere with DNA repair following radiation exposure [87]. The mechanism of CDK4/6 inhibitor radiosensitization may be related to the induction of cancer stem cell differentiation, which increases the vulnerability of cells to radiation-induced cell death [88]. The possible clinical benefits of CDK4/6 blockade on radiotherapy sensitization need to be further explored.

Conclusions

Disregulated activation of the cyclin D-CDK4/6-INK4-Rb pathway is frequently observed in a range of tumor types, and CDK4/6 has emerged as a promising therapeutic target. As a monotherapy, further efforts to refine a clear patient selection strategy and understand the mechanisms of acquired resistance will facilitate further development of CDK4/6 inhibitors in other patients beyond HR+ breast cancer. Factors such as differing dosing schedules (once daily with palbociclib and ribociclib versus twice daily with abemaciclib) and side effect profiles (largely hematological adverse events with palbociclib and ribociclib versus gastrointestinal effects with abemaciclib) may further influence clinical decisions regarding how CDK4/6 inhibitors are used in individualized patient care. Addition of CDK4/6 inhibitors to a variety of established treatments has the potential to improve responses to other therapies and may help overcome treatment resistance; in particular, the combination of CDK4/6 inhibitors with endocrine therapy is already FDA approved, and is expected to further transform the treatment of HR+ breast cancer.

Role of the funding source

Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals Corporation.

Author contribution

Both authors participated in all stages of manuscript preparation, and read and approved the final version prior to submission.

Acknowledgments

The authors thank Abbie Saunders PhD and Nirmal Jethwa PhD for medical editorial assistance with this manuscript.

References


DeMichele A. Palbociclib (PD-0332991) in breast cancer. 12th International Congress on Targeted Anticancer Therapies 2014; oral presentation O4.2.