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Targeting CDK4/6 pathways and beyond in breast cancer

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ABSTRACT

Metastatic or advanced breast cancer (mBC/ABC) remains incurable despite many different systemic treatment options. Hormone receptor positive (HR+) disease represents the most common subtype in both early and advanced disease. A better understanding of the biology of this BC subtype, in particular regarding potential mechanisms of endocrine resistance, has led to the development of CDK4/6 inhibitors. All three selective CDK4/6 inhibitors, palbociclib, ribociclib and abemaciclib have shown to significantly improve progression-free survival (PFS) when combined to endocrine therapy as first-line treatment for patients with HR+/HER-2 negative ABC, who have progressed on or after adjuvant endocrine therapy. All three of them have also shown an improved PFS as 2nd line therapy for HR+/Her2 negative ABC. Their toxicity profile is favorable, with hematological toxicity (mainly neutropenia) being predominant, followed by diarrhea and fatigue. Quality of life has been maintained in the 1st line setting or improved in the 2nd line setting. Overall survival (OS) has been reported so far only in 2 out of 7 trials as first line therapy and the difference did not reach statistical significance.

In this article we review the biology of CDK signaling pathway and its inhibitors, preclinical and clinical data of all three investigated selective CDK4/6 inhibitors and their toxicity. We also discuss how these agents are being included in current international guidelines and future directions for these agents in other subtypes of breast cancer, in both advanced disease and early-stage disease.

1. Introduction

About 1,6 million women are annually diagnosed worldwide with breast cancer, which remains the commonest malignancy in women. Systemic treatment for early and advanced breast cancer is mainly driven by the biological characteristics of the disease, predominantly by expression of estrogen receptors (ERs), progesterone receptors (PRs) and human epidermal growth factor receptor 2 (HER-2). Despite many available treatment options, ABC/mBC remains incurable. The commonest type of early and advanced breast cancer is hormone receptor - positive (HR+)/Her2 negative, also known as Luminal-like subtype. For many years endocrine therapy has been the main systemic treatment approach for patients with Luminal-like ABC. However, its efficacy may be limited by the development of de novo and/or acquired resistance [1]. In recent years, new therapies have been developed, aiming to potentially overcome or at least delay the onset of endocrine resistance. Primary endocrine resistance is defined as a relapse while on the first 2 years of adjuvant endocrine therapy (ET) or progression of disease within first 6 months of first line ET for ABC [2-ref ABC2]. In contrast, secondary (acquired) endocrine resistance is defined as a relapse while on adjuvant ET but after the first 2 years, or a relapse within 12 months of completing adjuvant ET, or progression of disease $\geq$ 6 months after initiating ET for ABC.

Selective cyclin-dependent kinase (CDK) 4/6 inhibitors affect cell cycle progression to interrupt tumor growth and have been extensively investigated in preclinical and clinical studies, mainly in patients with HR+/HER2 negative ABC. It has been demonstrated that the addition of a CDK 4/6 inhibitor to endocrine therapy significantly improves PFS in comparison to endocrine therapy alone as 1st or 2nd line endocrine therapy of Luminal-like ABC [2,3]. The combination of endocrine therapy and a CDK 4/6 inhibitor is now included in most national and international treatment guidelines as a treatment option, but several questions remain unanswered: which patients need upfront combination treatment strategy and which patients may be safely treated with ET alone,

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leaving the combination for a 2nd line. So far, the lack of biomarkers has made this choice difficult.

In this review article we discuss preclinical and clinical evidence-based data of the use of CDK 4/6 inhibitors in all subtypes of ABC with special emphasis on the luminal-like subtype. Additionally, we review current inclusion of these agents in international guidelines for the management of this ABC subtype. Finally, we also discuss the toxicity profile and cost-effectiveness of CDK 4/6 inhibitors, and their future developments.

2. Biology of cyclin-dependent kinases and its inhibitors

2.1. Cyclin-dependent kinases in cell cycle regulation

In healthy, normal cells, a cell cycle progresses from G1 (first growth period), to S (DNA synthesis), G2 (second growth phase), and M phase (mitosis) (see Fig. 1) [4]. The process is controlled by many different proteins, including CDKs, cyclins and the retinoblastoma (Rb)-E2F signaling pathway [5]. The Rb protein is a tumor suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumor progression [6]. It has been demonstrated that Rb protein (pRb) is responsible for a major G1 checkpoint, blocking S-phase entry and cell growth. E2F represents an evolutionarily conserved family of transcription factors that functions in cell cycle control and helps to promote tumor development [7]. The overexpression of E2F activators could trigger quiescent cells to enter G1 phase, independently of growth factor stimulation and fuell the initial identification of important E2F target genes that are involved in DNA replication. Studies have shown that Rb cannot regulate all stages of the cell cycle alone. Moreover, when Rb is active, E2F transcription modules stay in a suppressed state, which induces the recruitment of repressive chromatin marks, histone modifiers, and chromatin remodeling proteins. This results in cell cycle blockade (Fig. 1) [8]. The CDKs-Rb axis is essential to cell cycle entry. The combination of CDK4/6 and cyclin D induces phosphorylation and inactivation of Rb [9] and releasing of E2F, which results in the alteration of transcription of genes that are involved in the cell cycle process and subsequent G1-S blockade (Fig. 1) [8]. Moreover, the combination of CDK4/6 and cyclin D also induces phosphorylation of the cell proliferation-specific transcription factor forkhead box M1 (FOXM1), furtherly inducing the expression of genes, which drive cell division and inhibit cellular senescence in a FOXM1−dependent manner [10]. The kinase activity of CDK 4/6 is inhibited by $p16^{INK4A}$ [11–13] (Fig. 1), and cyclin D is coordinated by a complex network such as ER/PR/androgen receptor (AR), nuclear factor kB (NF-kB), mitogen activated protein kinases (MAPKs), signal transducers and activators of transcription (STATs), Wnt/β-catenin and phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR [10].

Additionally, the combination of CDK2 and cyclin E is also involved in the phosphorylation of Rb (Fig. 1). CDK2 is active in S, G2 and M phases of the cell cycle in combination with cyclin E and cyclin A, respectively, while CDK1/cyclin A/B complex regulates the transition from G2 to M phase (Fig. 1) [8].

In cancer cells, the process of cell division is dysregulated, which results in uncontrolled growth of tumor cells and formation of a tumor. Many different mechanisms lead to the dysregulation of the cell cycle in cancer cells including hyperactivity of CDK4/6 which initiate a phosphorylation cascade that inactivates the Rb1 protein and dissociates the entire repressive complex [14]. Consequently, this enables E2Fs to recruit transcriptional activators and alter transcription of genes involved in cell cycle progression, DNA synthesis and DNA replication as described above.

2.2. CDK 4/6 inhibitors

So far, several CDK inhibitors have been explored as potential targeted agents in several types of cancers and assessed for pharmacokinetics, efficacy and safety in many clinical trials. The most extensively investigated one among the first-generation pan-CDK inhibitors was flavopiridol, which is an inhibitor of CDK 1, 2, 4, 6, 7 and 9. It showed activity in hematologic cell lines, but was not very effective in the treatment of patients with chronic lymphocytic leukemia [15,16] and was associated with unacceptably high rates of dose-limiting toxicities, including neutropenia, hyperglycemia,

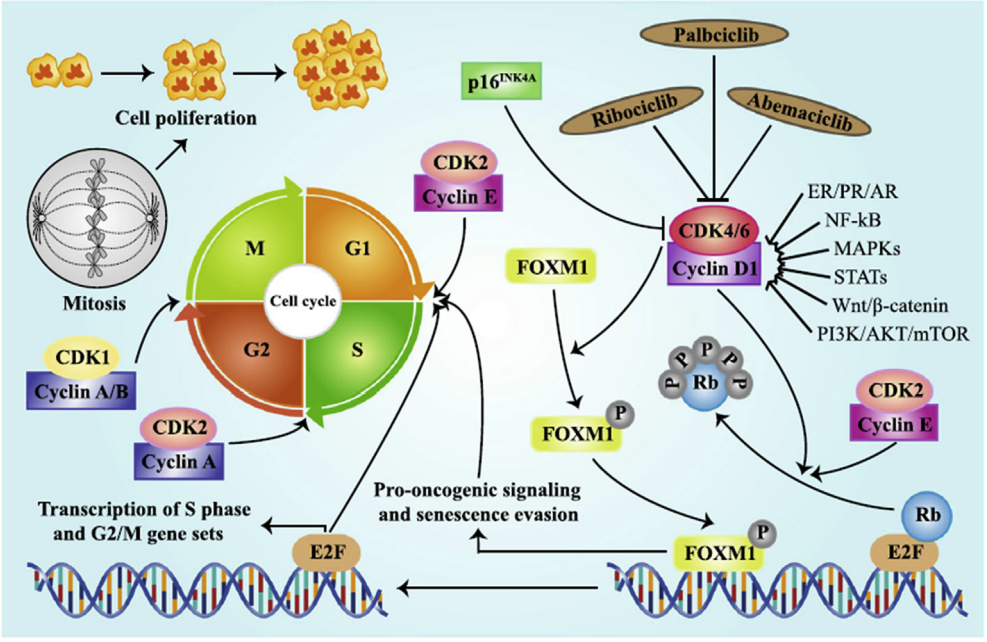

Fig. 1. Hanxiao X, Shengan Y, Qian L, Xun Y, Sridhar M, Richard GP et al. Recent advances of highly selective CDK4/6 inhibitors in breast cancer. Journal of Hematology and Oncology. 2017; 10 (97): 1–12.

cardiac and pulmonary dysfunction [17]. Subsequently, more potent and selective inhibitors targeting cyclin D1 were explored and developed. Among them, palbociclib, ribociclib and abemaciclib have been tested so far in clinical trials of luminal ABC (Table S1). They are all orally administered and generally, they all block the phosphorylation of Rb and related proteins, inducing downregulation of S phase cyclins and mitotic regulatory genes as well as suppressing nucleotide biosynthesis and DNA replication [18,19].

3. Preclinical data about CDK 4/6 inhibitors in various breast cancer subtypes

The Cancer Genome Atlas project revealed the heterogeneity of breast cancer (BC) in terms of molecular architecture of every subtype [20]. Focusing on retinoblastoma/RB1 pathway dysregulation, the mutation spectrum varies between BC subtypes (Table 2), providing a background for the development of targeted therapies, used either alone or in combination with other proven treatments for BC.

As cyclin D1 binds to CDK4 and CDK6 in order to phosphorylate the RB protein and to furthermore promote cell cycle progression from G1 to S phase, CDK4/6 became targetable in cancer research.

3.1. CDK 4/6 inhibitors in ER-positive breast cancer

In HR + breast cancers, there is extensive cross-talk between ER and cyclin D1 (CCND1) pathways, with cyclin D1 being a direct transcriptional target of the ER. The CCND1 oncogene is overexpressed in up to 50% of breast cancers [21] and it has also been suggested to be involved in endocrine resistance [22]. Various preclinical data suggested that CDK4/6 inhibitors could be effective in ER-positive and endocrine-resistant breast cancers.

One of the first molecules studied *in vitro* over a decade ago, *PD 0183812*, could lead to G1 cell cycle arrest when RB is expressed, targeting both CDK4 and CDK6 [23].

NPCD (Naphtho [2, 1-α] pyrrolo [3,4-c] carbazole-5, 7 (6H, 12H)-dione) has also been studied as a CDK4/6 inhibitor, inducing decreased Rb phosphorylation, G1-phase arrest and cell death in breast cancer cell lines [24].

Palbociclib (PD 0332991) was the first oral CDK4/6 inhibitor successfully implemented in clinical practice. The preclinical data showed similar effects as for other CDK4/6 inhibitors, in cell lines with detectable Rb protein. No activity of the molecule was observed in Rb-deficient cells [25], suggesting a dependency on the cyclin D1-CDK4/6-RB protein complex. While the Rb status is important in the short-term, a loss of Rb function associated with extended CDK4/6 inhibition can lead to palbociclib resistance, involving overexpression of CDK2, a cell cycle promoter, and the loss of inhibitory proteins such as p21 and p27 [26]. One *in vitro* study showed that the HR+/HER2 negative subtype seems to be more sensitive to the drug compared to other subtypes. Her2-positive cell lines that responded to palbociclib have luminal features [27]. Preclinical data also showed palbociclib to be an effective treatment in endocrine resistant disease [28], both in monotherapy but mostly in combination with endocrine agents [29]. The synergistic effects of combining palbociclib with tamoxifen in ER-positive cell lines were shown, since palbociclib increases the sensitivity of the ER-positive cells to endocrine therapy [27]. During treatment, the inhibition of CDKs leads to activation of autophagy, as a mechanism for treatment resistance [30] and the addition of an autophagy inhibitor to a CDK4/6 inhibitor can significantly reverse tumor resistance, decrease the dose of palbociclib required to treat breast cancer patients, with a possible significant impact on toxicity and quality of life. Moreover, Rb and cyclin E initially seemed to be predictive markers for response to the combination but were later not confirmed in clinical trials. Acquired resistance to palbociclib may also be linked to a PI3K- dependent upregulation of cyclin D1 [31]. *In vitro* studies suggest possible benefit from combining endocrine therapy and CDK4/6 inhibitor to a PI3K/mTOR inhibitor, by inducing a different mode of cell arrest and preventing the development of tumor resistance.

Ribociclib (LEE011), another selective CDK4/6 inhibitor approved for clinical use, has been tested in preclinical models, using various cancer cells such as liposarcoma, lung, breast and neuroblastoma [32,33]. Although with similar mechanisms of action, the resistance mechanisms seem to differ between ribociclib and palbociclib [14], with an increased E2F1 in ribociclib cells compared with increased cycle E protein in palbociclib-resistant cells. Through noncanonical cyclin D1-CDK2 mediated S-phase entry [31], both palbociclib and ribociclib resistant clones seem to confer cross-resistance to both drugs [34]. Preclinical data showed that in tumor cells with altered PI3K pathway, the use PI3K inhibitors alone is not effective in inducing apoptosis. However, the use of additional CDK4/6 inhibitors can sensitize resistant cells, increasing the effect of a PI3K inhibitor [35]. Based on these observations, the combination of a CDK4/6 inhibitor and a PI3K inhibitor is being tested in clinical trials (NCT0185719319 and TRINITI-120). Since PI3K/AKT/mTOR pathway has been implicated in treatment resistance, the combination of ribociclib and a PI3K inhibitor, alpelisib, was evaluated and showed increased response rates and PFS compared to single agents in patient-derived xenograft mouse models [36].

Abemaciclib (LY2835219), a reversible selective CDK4/6 inhibitor had similar class effects as the other agents, namely inhibition of RB phosphorylation and G1 cell cycle arrest in a concentration-dependent manner [37]. Both senescence and apoptosis occurred at lower concentrations compared to palbociclib or ribociclib. Preclinical data suggest that both palbociclib and ribociclib resistant clones confer incomplete cross-resistance with abemaciclib [31]. Inhibition of CD4/6-Rb-E2F pathway not only induces tumor cell-cycle arrest, but also promotes an anti-tumor immune response through antigen presentation in tumor cells and T-cell activation [38]. Treatment combination with abemaciclib and anti-PD-L1 −based immunotherapy was effective in Rb-competent breast cancer cells, due to enhancement of both immunomodulatory and cell cycle suppression effects [39].

3.2. CDK 4/6 inhibitors in HER-2-positive breast cancer

HER-2-positive breast cancer bears a worse prognosis in untreated patients in terms of survival compared to the luminal-like subtype [40], and CDK4 overexpression may also play a role in predicting the occurrence of early brain metastases [41]. In these tumor cells, there are frequent alterations in the PI3K/Akt pathway, the signaling mediator Akt acting as a negative regulator of the CDK inhibitor $p57^{Kip2}$. As p57 acts as a tumor suppressor gene by negatively regulating the cell cycle, its decreased expression is correlated with increased cell proliferation [42].

Palbociclib proved to be effective in inhibiting growth in Her2-positive cell lines. The efficacy of CDK4/6 inhibitors in this case could be explained by a subset of cells, which also have high cyclin D1 levels. However, as synergistic effects on tumor cells between palbociclib and trastuzumab have been recorded, other mechanisms, independent of cyclin D1 levels, may be involved [27].

3.3. CDK 4/6 inhibitors in other settings

As preclinical data come in favor of the combination of CDK4/6 inhibitors and other targeted agents, their addition to standard chemotherapy is still being investigated. Current results are not

favorable to this approach, as various *in vitro* studies that included a CDK4/6 inhibitor and cytotoxic agents such as taxanes [43], platinum agents [44] or anthracyclines [45] suggest a possible interference between cytostatic and cytotoxic mechanisms, although the sequential use of these agents could be considered. Therefore, combining a CDK4/6 inhibitor with agents that exert their cytotoxic effects on cell proliferation needs to be carefully evaluated.

In triple negative breast cancer, functional loss of Rb has been frequently described [46], inducing cancer proliferation by activation of mitochondrial protein translation (MPT) gene pathway [47]. Although Rb deficiency has been associated with sensitivity to some chemotherapeutic agents in preclinical models, these cells are resistant to CDK 4/6 inhibitors.

4. Clinical data about CDK 4/6 inhibitors in various breast cancer subtypes

Currently there are many phase II and phase III ongoing clinical trials with the three main CDK 4/6 inhibitors (palbociclib, ribociclib and abemaciclib) in patients with early-stage and advanced breast cancer (see Table S1).

4.1. Palbociclib

4.1.1. Phase I data

In the first-in-human phase I study evaluating the role of palbociclib in 33 patients with Rb-positive metastatic solid tumors or refractory non-Hodgkin's lymphoma, 1 patient with advanced testicular cancer achieved a partial response and 9 patients had stable disease [48]. Palbociclib was given once per day for 14 days and one week off. Toxicity profile was mild to moderate with myelosuppression being the most common side effect. The maximum tolerated dose was 200 mg once daily. In a second phase I dose escalation study, 41 patients with metastatic Rb-positive solid tumors received palbociclib in a 3 weeks on/1 week off scheduling [49]. 10 (27%) patients achieved stable disease for ≥ 4 cycles and 6 of them derived prolonged benefit ($\geq$10 cycles). The dose of 125 mg was found to be the one that caused dose-limiting toxicity that was myelosuppression including grade 3 neutropenia and anemia. Other, non-hematological toxicity was mild and included mainly fatigue, diarrhea and nausea.

4.1.2. Phase II data

Palbociclib as a single agent was investigated in a phase II trial that included 36 heavily pretreated patients with Rb-positive metastatic breast cancer at a dose of 125 mg once daily, days 1–21 out of a 28 days cycle [50]. 28 patients completed treatment, with 4 of 18 patients with HR+/HER-2 negative subtype of disease having for 6 months or more. The main toxicity was myelosuppression leading to treatment interruptions in 25% of patients.

Another randomized phase II trial (TREND trial) [51] investigated palbociclib as a single agent vs. palbociclib in combination with endocrine therapy in 115 pretreated patients. The clinical benefit rate (CBR) was 54% for combination treatment (95% CI 41.5–63.7) and 60% for monotherapy (95% CI 47.8–72.9), with a median PFS of 10.8 months (95% CI 5.6–12.7) for combination therapy and of 6.5 months (95% CI 5.4–8.5) for monotherapy. The exploratory analysis revealed no PFS advantage for the combination in patients who received prior endocrine therapy less than 6 months, suggesting the potential benefit of palbociclib in reversing endocrine resistance.

Based on these results and the findings from preclinical studies, the open-label phase II PALOMA-1 trial was conducted. 165 postmenopausal women with metastatic HR+/HER-2 negative breast cancer that had not received any systemic therapy for their advanced disease were randomized to either letrozole alone or in combination with palbociclib at a dose of 125 mg 3 weeks on and 1 week off scheduling [52]. Patients were enrolled in two cohorts, 66 patients in the first cohort were randomized without selection by potential predictive biomarkers, whereas tumors from the 99 patients in the second cohort had to be positive for either cyclin D1 amplification, loss of p16 or both. There was a statistically significant difference in median PFS between treatment arms: 10.2 months (95% CI 5.7–12.6) for the letrozole arm and 20.2 months (95% CI 13.8–27.5) for the combination arm. This improvement was independent of the presence of cyclin D1 amplification or p16-loss, not confirming their role as potential biomarkers. Median overall survival (OS) was 37.5 months in the combination arm and 33.3 months in the letrozole alone arm (HR = 0.935; $P = 0.388$), which was not significantly different [53]. The most common adverse event was neutropenia; grade 3–4 neutropenia was observed in 45 (54%) of 83 patients in the combination arm in comparison to 1 (1%) of 77 patients in the letrozole alone arm. Importantly, there were no cases of febrile neutropenia reported. Fatigue was more common in the combination arm (4% versus 1% for grade 3–4). Based on the results of this trial, the FDA provided accelerated approval of palbociclib in combination with letrozole in February 2015.

4.1.3. Phase III data

With the aim to further validate and confirm the findings of the PALOMA-1 trial, a phase III, double blind placebo-controlled randomized PALOMA-2 trial, was conducted in the first-line metastatic setting. It enrolled 666 postmenopausal women with previously untreated HR+/HER-2- negative ABC who were randomly assigned, in a 2:1 ratio, to palbociclib and letrozole versus letrozole alone [54]. The primary endpoint was PFS, as assessed by the investigators. Around 50% (48.6%) of all included patients had visceral metastases, 63% had received prior systemic therapy, 37% had de novo metastatic disease and 22% had a disease-free interval (DFS) of 12 months or less. After a median follow-up of 23 months, the median PFS was 24.8 months (95% CI 22.1-not estimable) in the palbociclib plus letrozole group in comparison to 14.5 months (95% CI 12.9–17.1 months) in the placebo plus letrozole group (HR 0.58; 95% CI 0.46–0.72, $p < 0.001$). The most common toxicity in the palbociclib arm was hematological with 66% of grade 3 or 4 neutropenia, 25% of leukopenia, 5% of anemia and 2% of fatigue in comparison to 1%, 0%, 2% and 0.5% in the placebo arm, respectively. The rate of febrile neutropenia was 1.8% in the palbociclib arm versus 0% in the placebo arm. Additionally, alopecia was more frequently reported in the palbociclib group (30% vs. 15% grade 1; 3% vs. 1% grade 2). Health-related quality of life was well maintained on treatment with palbociclib and there were no meaningful differences seen with the placebo arm [55]. However, 33 patients (7%) in the palbociclib arm permanently stopped the treatment due to adverse events (AEs) in comparison to 10 patients (4.5%) in the placebo arm. The initial report from the Paloma 2 trial [56] did not show an improved health related quality of life with the addition of Palbociclib to letrozole vs. letrozole alone (FACT- G score of −0.39 vs −0.53; $p = 0.882$ and FACT-B score of −0.11 vs 0.22; $p = 0.782$). However, a more recent study [57] suggests that increased PFS was associated with delayed deterioration of quality-of-life both in the palbociclib (HR: 0.53; 95% CI 0.38–0.73; 1 −sided p-value < 0.001) and in the placebo arms (HR:0.57; 95% CI 0.35–0.91; 1 −sided p-value = 0.009).

A second phase III trial, PALOMA-3, evaluated the combination of fulvestrant and palbociclib in comparison to fulvestrant and placebo in 521 pre- and postmenopausal women with HR + HER-2-negative ABC who had progressed during previous endocrine therapy [58]. In premenopausal patients, ovarian function suppression (OFS) with an LHRH analogue goserelin was added. After a

preplanned interim analysis, the study was stopped due to a significant difference in PFS favoring the palbociclib arm. A second analysis of efficacy after a median follow-up of 8.9 months confirmed the substantial difference of PFS with a median PFS of 9.5 months (95% CI 9.2–11.0) in the palbociclib arm versus 4.6 months (95% CI 3.5–5.6) in the placebo arm (HR 0.46; 95% CI 0.36–0.59; $p < 0.0001$) [3]. After a median follow-up of 15 months, median PFS was 11.2 months vs. 4.6 months (HR 0.497; 95% CI 0.398–0.620; $p < 0.0001$) [59]. This study was the first phase III trial showing a benefit with the combination of palbociclib in premenopausal women rendered postmenopausal with the addition of OFS with goserelin. Toxicity was similar to that observed in the PALOMA-2 trial, with grade 3 or 4 hematological AEs being reported more common in the fulvestrant-palbociclib group (73% versus 22%). Neutropenia grade 3 or 4 occurred in 65% of patients in the fulvestrant-palbociclib arm. A detailed analysis demonstrated that Asian patients and those with below-median neutrophil counts at baseline had a significantly increased risk of developing grade 3 or 4 neutropenia with palbociclib and importantly, there was no adverse impact seen on PFS with the dose reduction needed for grade 3–4 neutropenia [60]. Patient-reported outcomes from PALOMA-3 trial showed that quality of life (QoL) scores favored the combination arm (estimated overall global QoL scores significantly favored the palbociclib arm (66 versus 63, $p = 0.0313$), with a significant delay in deterioration of QoL with palbociclib (HR: 0.641; 95% CI: 0.451–0.910; $P = 0.0065$) that corresponds to the delay in disease progression [61].

Palbociclib is currently being evaluated in many neoadjuvant and adjuvant phase II and III clinical trials that are in a recruiting phase (see Table S1).

4.2. Ribociclib

4.2.1. Phase I-II data

In a phase I trial, 132 patients with metastatic solid tumors and refractory lymphomas were treated with ribociclib, with doses of 70–1200 mg daily for 21 of 28 days and drug activity was noticed at doses of ≥600 mg, with the maximum tolerated dose of being 900 mg [62]. 2 patients had partial response and one of them had ER-, PIK3CA mutation- and cyclin D1 amplification positive breast cancer and the other had BRAF and NRAS wild type and cyclin D1 amplification positive malignant melanoma. Stable disease was reported in 26% and 14% for ≥4 and ≥ 6 cycles in the whole group, respectively. Most commonly identified toxicities were myelosuppression (neutropenia, leukopenia, asymptomatic thrombocytopenia), diarrhea, nausea and asymptomatic QTc prolongation.

Ribociclib has been investigated in phase Ib/II trials in combination with endocrine agent exemestane and mTOR inhibitor everolimus and in combination with letrozole and a PI3Kα inhibitor alpelisib (see Table S1). Additionally, based on preclinical results [63], phase Ib/II clinical trials have been opened to evaluate ribociclib in combination with trastuzumab or trastuzumab-emtansine (T-DM1) for patients with HER-2 positive locally advanced or metastatic disease.

A phase II, multicenter, randomized trial evaluated the safety, pharmacokinetics and pharmacodynamics of two clinical doses of ribociclib (400 mg and 600 mg) in combination with letrozole as presurgical treatment in HR + early breast cancer [64]. 14 premenopausal patients were randomized to one of three treatment arms: letrozole 2.5 mg/day (Arm 1), letrozole and ribociclib 400 mg/day (Arm 2) or letrozole and ribociclib 600 mg/day (Arm 3). Tumor biopsy and blood samples were collected during treatment in order to assess Ki67 expression, pRb level and circulating tumor DNA (ctDNA). No grade 3/4 toxicity was observed and most commonly identified toxicities were nausea, asthenia, QT prolongation and decreased appetite in Arms 2 and 3. Ki67 levels mean percent decrease was 96% in Arm 2 (range 78–100%; $n = 6$) and 92% in Arm 3 (range 75–100%; $n = 3$). pRb levels increased from baseline to day 15 in Arm 1, while in Arms 2 and 3 pRb levels decreased in five out of eight evaluable patients, with a mean percent decrease in pRb levels of 59% (range 31–95%; $n = 5$).

4.2.2. Phase III data

The results of a phase III randomized double blind, placebo-controlled trial, MONALEESA-2, have been published [65,66]. In this trial, 668 post-menopausal women with HR+/HER-2-negative ABC, who had not had any previous systemic therapy for their advanced disease, were randomized between ribociclib and letrozole or placebo and letrozole. The dosing and scheduling of ribociclib was 600 mg daily on a 3 weeks on and one week off basis. The primary endpoint was PFS assessed by investigators. After a median follow-up of 26.4 months, the median PFS was 27.6 months vs. 15 months (HR, 0.527; 95% CI, 0.351–0.793), favoring the combination arm. Most frequent grade 3 or 4 AEs included neutropenia (59%), leukopenia (21%), hypertension (10%), followed by increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (9% and 6%, respectively). Febrile neutropenia occurred in 5 patients (1.5%) in the ribociclib group and in none in the placebo group. Ribociclib was approved by FDA and EMA in 2017 based on these results.

The efficacy of ribocliclib for premenopausal patients was studied in a phase III randomized double blind trial, MONALEESA-7 [67]. In this trial, 672 patients with HR+/HER-2-negative ABC were randomized between ribociclib (600 mg daily on a 3 weeks on and 1 week off schedule) or placebo with either tamoxifen (20 mg daily) or a non-steroidal aromatase inhibitor (letrozole 2·5 mg or anastrozole 1 mg daily), all with goserelin (3·6 mg every 4 weeks). After a median follow-up of 19.2 months, the median PFS was 23.8 months vs. 12 months (HR 0·55, 95% CI 0·44–0·69; $p < 0.0001$), favoring the combination arm. It is important to note that 40% of patients had de novo ABC and 57% had visceral metastases. The quality of life sub-study showed a sustained improvement in time to definitive deterioration in the ribociclib arm. The adverse-events profile was consistent with the class effects of CDK4/6 inhibitors, and there was no added toxicity with the use of goserelin. A greater proportion of patients in the ribociclib arm had a prolongation of the QTcF interval (10% vs.4%), without clinical symptoms or arrhythmias, with a higher incidence in patients receiving tamoxifen compared to those receiving letrozole or anastrozole (16% vs. 7%).

4.3. Abemaciclib

Another CDK4/6 inhibitor that has shown promising results in chemotherapy-naïve, ER-positive/HER2-negative mBC patients after progression while on endocrine therapy is abemaciclib, as was demonstrated in the MONARCH-2 trial [68] (PFS 16.4 months vs. 9.3 months; $HR = 0.55$), and data that were also supported by MONARCH-3, a phase III trial [69]. A subgroup analysis of MONRACH-2 trial, performed in peri/pre-menopausal patients, has shown a similar magnitude of benefit for this subgroup as for the ITT population. No additional toxicities were observed by adding a GnRH agonist to abemaciclib plus fulvestrant. The efficacy of abemaciclib as monotherapy was also suggested by MONARCH-1 trial [70], in heavily pretreated ER-positive/HER2-negative endocrine resistant patients, who reached the point where chemotherapy would be indicated. Single agent abemaciclib provided a median PFS of 6 months and a median OS of 22.3 months, after 18 months follow-up. These data may suggest that for heavily pretreated endocrine resistant patients, single agent abemaciclib may be an option, as chemotherapy is frequently associated with increased

toxicity and decreased quality of life. However, further data comparing CDK4/6 inhibitors in monotherapy vs. chemotherapy alone are needed to be able to answer this question. From a preclinical point of view, the efficacy of abemaciclib was observed at lower doses compared to the other CDK4/6 inhibitors [37], and therefore the possibility of similar clinical outcome with decreased toxicity should be further researched. Slightly different compared to its class partners, since it is more selective to CDK4 than CDK6 [71] and can be taken on a continuous schedule.

Two phase III trials are investigating the drug in combination with endocrine therapy in first- and second-line setting (NCT02246621 and NCT02107703).

4.3.1. Phase I-II data

A phase I trial investigated the role of abemaciclib as single agent in patients with various metastatic solid tumors (with an emphasis on ABC) [72] using the dose of 150–200 mg, twice daily, in a 28-day cycle. The dose-limiting toxicity was diarrhea, different from phase I trials of palbociclib and ribociclib. The diarrhea could be managed with the use of prophylactic antidiarrheal and with interruptions/reductions as needed. Other toxicities included neutropenia, nausea, vomiting and fatigue. Uncomplicated neutropenia was the most common adverse event among grade 3 toxicities. In this trial, 47 patients with ABC progressing after several lines of systemic therapy were enrolled and in 23 (49%) a clinical benefit was seen. In the subgroup of patients with HR + ABC, 25% achieved partial response and 61% clinical benefit. Based on these findings, this phase I trial was expanded to evaluate the role of fulvestrant and abemaciclib in patients with HR + ABC [73]; 18 additional patients received abemaciclib 200 mg twice daily with fulvestrant, in a 28-day cycle, 72% of which experienced clinical benefit. The toxicity profile for combination therapy was similar to the one observed with single agent abemaciclib.

4.3.2. Phase III data

The results of the phase III randomized, double blind placebo-controlled, MONARCH-2 trial have been published [74]. In this trial, 669 pre- and postmenopausal women with HR+/HER-2 negative ABC, who had progressed on (neo)adjuvant endocrine therapy, $\leq$ 12 months from the end of adjuvant endocrine therapy or on first line hormonal treatment for advanced disease who had not received chemotherapy for metastatic disease, were randomly assigned (2:1) to receive abemaciclib at a dose of 150 mg $q12^h$ or placebo plus fulvestrant and stratified by metastatic site (visceral, bone only, other) and resistance to prior endocrine treatment (primary versus secondary). All pre- and perimenopausal patients received OFS with gonadotropin-releasing hormone agonist. The primary endpoint was PFS, assessed by investigators. 56% of patients had visceral disease, 72% measurable disease and 25% had primary endocrine resistance. Median PFS was 16.4 months for abemaciclib and fulvestrant combination vs. 9.3 months for placebo and fulvestrant (HR 0.553; 95% CI 0.449–0.681, p = 0.001). The most common AEs were diarrhea (86% vs 25%), neutropenia (46% vs 4%), nausea (45% vs 23%), and fatigue (40% vs 27%). The rate of neutropenia was lower than with other CDK4/6 inhibitors. This finding may be explained by the greater relative potency of abemaciclib for cyclin D1/CDK4 compared with cyclin D3/CDK6 observed in cell-free enzymatic assays [75].

The MONARCH-3 trial [76] evaluated abemaciclib in combination with letrozole in 493 post-menopausal patients as 1st line treatment for metastatic breast cancer. Patients were randomized to either the combination treatment or letrozol and placebo and stratified by metastatic site and prior endocrine treatment. The main endpoint was PFS, secondary endpoints including ORR and safety. The study showed a significantly improved PFS (HR = 0.543, 95%CI, 0.409 to 0.723, p = 0.000021). Median PFS was 14.7 months in the letrozol arm and was not yet reached in the combination arm. Most common AEs were similar to MONARCH-2 trial, the most frequent being diarrhea (81.3% vs. 29.8%), neutropenia (41.3% vs. 1.9%) and fatigue (40.1% vs. 31.7%).

Abemaciclib was approved by FDA in September 2017 and its approval in Europe is foreseen for early 2018.

Table S1 includes currently ongoing phase II and phase III clinical trials evaluating the role of CDK inhibitors in combination with different agents in patients with early-stage and advanced breast cancer. Table 1 details the main phase III clinical trials with the three selective CDK4/6 inhibitors (palbociclib, ribociclib and abemaciclib).

4.4. CDK inhibitors in neoadjuvant setting

Due to their favorable results in the metastatic setting, these drugs are now being tested in the neoadjuvant or/and adjuvant settings. Neoadjuvant treatment combinations with endocrine therapy have reported an overall response rate (ORR) of 89% and a pathological complete response (pCR) of 11% [77]. The NeoPAL study [78], exploring the role of neoadjuvant palbociclib and endocrine therapy in luminal-like early BC, included 106 patients randomized after risk of recurrence (ROR) score defined by PAM50 assay to either endocrine therapy combined with palbociclib or to chemotherapy. Clinical response rate (74.5% vs. 76%) and breast conserving surgery rate (69.2% vs. 68.6%) were similar in the two groups, with less toxicity in the endocrine therapy + palbociclib arm.

For patients with residual disease after neoadjuvant treatment, the combination of palbociclib and endocrine therapy is currently being investigated in the PENELOPE-B trial (NCT18644746). Adjuvant long-term (two years) CDK4/6 and endocrine therapy is also currently being researched in the PALLAS trial (NCT02513394). As seen with other therapies, using CDK4/6 inhibitors both in early and in first lines setting for advanced disease generally leads to development of treatment resistance, so identifying patients that would benefit the most from this treatment is essential, as well as identifying solutions for when resistance occurs.

Given the success of CDK4/6 inhibitors in ER-positive tumors and also given some preclinical data showing some activity in HER2-positive cell lines [27], testing the efficacy of these agents in HER2-positive or ER-negative tumors was tested in neoadjuvant setting and is currently ongoing for advanced disease. In neoadjuvant setting, the NA-PHER2 phase II trial [79] combined trastuzumab, pertuzumab, palbociclib and fulvestrant in 35 patients with ER-positive/HER2-positive tumors. Pathologic complete response in breast and axillary nodes was obtained in 27% of the patients (95% CI 12–46, n = 8) of the patients, with manageable toxicity profile. The PATRICIA phase II trial [80] combines palbociclib and trastuzumab with or without letrozole in HER2+ advanced breast cancer using a Simon 2-stage design (NCT02448420). In this trial, 31 pretreated patients were included and further characterized according to PAM50. Compared to non-luminal-like disease, luminal-like subtypes had a longer PFS (10.37 vs. 3.53 months, p = 0.033), with a HR = 0.34 (95%CI 0.13–0.92,p = 0.033).

As CDK4 overexpression in HER2-positive tumors seems to predict early occurrence of brain metastases [41], further research in patients with HER2-positive disease is needed. One phase two, Simon 2-stage study [81] is exploring the efficacy of abemaciclib in patients with brain metastases, as it has shown preclinically to be able to cross the blood-brain barrier. So far, 23 patients were included in the first stage of the study, with an 8.7% of partial response, providing preliminary evidence that abemaciclib has antitumor efficacy in patients with brain metastases. Further

Table 1
Main characteristics and findings of phase III clinical trials with palbociclib, ribociclib and abemaciclib.

	PALOMA 2	PALOMA 3	MONALEESA 2	MONARCH 2	MONALEESA-7
Design	double blind, placebo-controlled (2:1 ratio)	double blind, placebo-controlled	double blind, placebo-controlled	double blind, placebo-controlled	double blind, placebo-controlled
Patient population	Postmenopausal patients 1st line ABC Previously treated/ untreated ECOG 0-2	Any menopausal status 1st/ 2nd line ABC Previously treated ECOG 0-1	Postmenopausal women 1st line ABC Previously treated ECOG 0-1	Any menopausal status 1st/ 2nd line ABC Previously treated ECOG 0-1	Premenopausal women 1st line ABC Previously treated ECOG 0-1
Number of patients	666	521	668	669	672
CDK4/6 inhibitor tested	palbociclib	palbociclib	ribociclib	abemaciclib	ribociclib
Endocrine combination therapy	letrozole	fulvestrant	letrozole	fulvestrant	letrozole/anastrozole or tamoxifen
Primary endpoint	PFS	PFS	PFS	PFS	PFS
Overall survival	Not reported	Not reported	Not reported	Not reported	Not reported
QoL study	No difference between arms	Delayed deterioration with palbociclib	No difference between arms	Not reported yet	Delayed deterioration with ribociclib
Median PFS (CDK4/6 inh vs control)	24.8 vs 14.5 months	9.5 vs 4.6 months	Not reached vs 14.7 months	16.4 vs 9.3 months	23.8 vs. 13 months
Hazard ratio	0.58	0.46	0.56	0.55	0.55
ESMO-MCBS	3	4	3	3	4
Reference	2	3	65	74	67

PFS – progression-free survival.

Table 2
Molecular alterations involving the RB pathway according to subtype [20].

	Luminal A	Luminal B	Basal-like	Her2-positive
1. Cyclin D1 amplification	49%	58%	9%	38%
2. CDK4 gain	14%	25%	–	24%
3. Expression of CDKN2C	Low	–	High	–
4. Expression of RB1	High	–	Low	–

CDK – cyclin-dependent kinase; RB1- retinoblastoma, Her2-human epidermal growth factor receptor.

research is needed to confirm the findings in this important and underserved subgroup of patients.

Triple negative breast cancer, characterized generally as an aggressive phenotype and absence of ER, PR and HER2 receptors, remains a heterogeneous group of diseases, with a paucity of treatment options. However, recent preclinical data have suggested a susceptibility of the luminal androgen receptor (LAR) subtype of TNBC to CDK4/6 inhibitors, especially in overcoming anti-androgen resistance [82]. In order to address this question, a phase II trial assessing the combination of palbociclib and bicalutamide for AR-positive metastatic TNBC is currently ongoing (NCT02605486).

5. The ESMO magnitude of clinical benefit scale (ESMO-MCBS) for CDK inhibitors and their incorporation in international guidelines

The ESMO-MCBS [83] is a validated tool used to objectively assess the benefit provided by new cancer therapies, offering a relative ranking of the magnitude of benefit from a given treatment in a given indication and setting.

For the non-curative setting, the scale provides a score between 1 (lowest benefit) and 5 (highest benefit). Depending on the primary endpoint of the trial, different forms are to be used. If only PFS benefit is seen, a score of 5 cannot be obtained. After grading for efficacy, the scale mandates an evaluation of quality of life; if a treatment improves quality of life the score may be increased by 1 point, but if it does not improve quality of life neither provides an OS benefit, then the score is downgraded by 1 point.

All three CDK4/6 inhibitors, when used in the 1st line setting (based on the efficacy results of Paloma-2, Monaleesa-2 and Monarch-2 trials), reach a score of 3 for efficacy. Since no OS benefit or improvement in quality of life was yet seen in this setting, the final score is a 3.

Palbociclib use in the 2nd line setting reaches a score of 4 for the efficacy results of Paloma-3 trial; since it also leads to an improvement of quality of life, the score is upgraded 1 point (final score: 4, pending OS results).

Abemaciclib use in the 2nd line setting has a provisional score of 3 based on the efficacy results of Monarch-3, and waiting for the results of the quality of life and OS analyses.

Based on these results, the ESO-ESMO ABC Consensus Guidelines considers the addition of a CDK4/6 inhibitor to an aromatase inhibitor, in naïve patients or pre-exposed to endocrine therapy, one of the preferred treatment options [84]. Patients relapsing less than 12 months from the end of adjuvant aromatase inhibitor were not included in the published studies and may not be suitable for this combination [84]. In patients previously exposed to an aromatase inhibitor, the addition of a CDK4/6 inhibitor to fulvestrant, is considered by the ABC Guidelines one of the preferred treatment options, if a CDK4/6 inhibitor was not previously used [84].

NCCN guidelines (version 2.2017) suggest the use of palbociclib or ribociclib in combination with letrozole as a treatment option for first-line therapy for postmenopausal patients with HR+/HER-2 negative metastatic breast cancer [85]. Premenopausal patients with HR+/HER-2 negative advanced disease should be given OFS with gonadotropin-releasing hormone analogue if they are planned to be treated with palbociclib or ribociclib in combination with letrozole. Those postmenopausal women or those premenopausal women receiving OFS with an LHRH agonist, with HR+/HER-2 negative metastatic breast cancer that have progressed on or after prior adjuvant or first-line endocrine therapy, could be offered the combination of palbociclib and fulvestrant. Importantly, if there is further disease progression while on a CDK4/6 inhibitor and letrozole, there are no evidence-based data to support an additional line of CDK4/6 inhibitor [85].

ASCO guidelines recommend the addition of palbociclib to a nonsteroidal AI may be offered to postmenopausal women with treatment-naïve HR-positive MBC, because progression-free survival (PFS) but not overall survival (OS) was improved compared with letrozole alone. In addition, fulvestrant and palbociclib may be offered to patients who experienced progression during prior treatment with AIs with or without one line of prior chemotherapy, because PFS was improved compared with fulvestrant alone. Only patients without prior exposure to cyclin-dependent kinase 4/6 inhibitors should be offered this combination [86].

6. Biomarkers

As with all therapeutic agents in Oncology, not all patients need to be treated or derive significant benefit from CDK 4/6 inhibitors. The identification of predictive biomarkers for treatment response is of crucial importance, since it would allow costs savings and spare patients who do not benefit from unnecessary toxicities. However and so far, no clinical predictors (such as visceral vs non-visceral disease, disease-free interval and luminal-A-like or luminal-B-like disease) have been identified that would clearly demonstrate which patients will benefit from the addition of CDK4/6 inhibitors and which will not [87].

In addition, there are also currently no known tissue or blood biomarkers that would predict benefit from CDK inhibitors. A biomarker analysis showed that neither amplification of cyclin D1, loss of p16, or PIK3CA mutational status nor hormone-receptor expression level affected response to combined therapy with palbociclib and endocrine agents [3,52]. Preclinical data suggested a positive correlation between cyclin D and Rb protein with *in vitro* response, as well as a negative correlation with p16 expression [25–27]. Unfortunately, the prospective evaluation of these biomarkers in the phase II Paloma-1 trial (Rb localization, Ki67 levels, p16 expression or cyclin D1 amplification) did not show any significant correlation with clinical or PFS benefit [88]. However, another study of single agent palbociclib suggested that increased Rb levels and a low Ki67 or a loss of p16 expression might be linked to response [89]. The real significance of Ki67 levels and p16 expression is still under study and some ongoing trials (NCT01976170; NCT01320592) only enroll patients with Rb overexpression. Extensive biomarker analysis in the PALOMA-2 trial also did not reveal any additional useful biomarkers except ER positivity that could indicate an increased sensitivity to CDK4/6 inhibitors [90].

7. Conclusions and future directions

Metastatic/Advanced BC is still an incurable disease and when evaluating new treatment options for affected patients it is crucial to consider not only efficacy but also their toxicity and impact in overall quality of life.

Improved knowledge of endocrine resistance in HR+/HER-2 negative ABC has helped to develop inhibitors of cyclin-dependent kinases 4/6. Selective CDK4/6 inhibitors, palbociclib, ribociclib and abemaciclib have been found to be effective in the treatment of this ABC subtype by significantly improving progression-free survival when combined to endocrine agents, both in the 1st and 2nd settings.

While the emergence of CDK4/6 inhibitors is changing the current clinical practice landscape for Luminal-like ABC, many questions remain: a) OS results are still awaited and may change the clinical implementation of these agents; b) the best sequencing of all available treatment options for this subtype of ABC is currently unknown; c) how do combinations of CDK4/6 inhibitors + endocrine therapy and mTOR inhibitors + endocrine therapy compare with each other and with chemotherapy is also unknown; d) the mechanisms of resistance to CDK inhibitors need further research and the best treatment after progression on CDK inhibitors needs to be identified.

Despite the scarce or conflicting preclinical data, combining CDK4/6 inhibitors with other approved treatments such as chemotherapy, radiation therapy or immunotherapy should be further investigated in clinical trials. Taking into account the heterogeneity of breast cancer subtypes, these approaches may be applicable in certain subgroups of patients. As each type of breast cancer treatment comes with different side effects, indicators such as quality of life, toxicity profile and drug-drug interactions should be taken into account when designing these clinical trials.

The development of adequate tools to evaluate quality of life in the advanced setting will allow for an accurate assessment of the impact of these new agents in the quality of life of ABC patients, considering its higher toxicity when compared to endocrine therapy alone, as well as the need for closer monitoring mainly of hematological parameters.

Finally, further biomarker analysis studies are indispensable to better select patients who derive the greatest benefit from CDK4/6 inhibitors, in both early and advanced settings. The costs of these new agents limit their affordability and accessibility for many ABC patients around the world and make their use in an unselected breast cancer population, unsustainable in the majority of countries.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2018.10.001.

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