Specific CDK4/6 inhibition in breast cancer: a systematic review of current clinical evidence

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ABSTRACT

Background Loss of cell cycle control is a hallmark of cancer, and aberrations in the cyclin-dependent kinase-retinoblastoma (CDK-Rb) pathway are common in breast cancer (BC). Consequently, inhibition of this pathway is an attractive therapeutic strategy. The present review addresses efficacy and toxicity of CDK4/6 inhibition in BC.

Methods A literature search was carried out using PubMed and EMBASE; data reported at international meetings and clinicaltrials.gov were included.

Results Three specific CDK4/6 inhibitors palbociclib, abemaciclib and ribociclib are tested in clinical trials. A randomised phase II trial of palbociclib plus letrozole versus letrozole and a phase III of palbociclib plus fulvestrant versus fulvestrant showed significantly increased progression-free survival when compared with endocrine therapy alone in first-line and second-line treatment for advanced hormone receptor-positive HER2-negative BC. At the moment several phase III studies are ongoing with all three CDK4/6 inhibitors in hormone receptor-positive HER2-negative BC as well as other subtypes of BC. The predominant toxicity of agents was limited neutropenia. Other common adverse events were infections, fatigue and gastrointestinal toxicity. The toxicities seemed manageable. Yet data are too limited to differentiate between the compounds. Retinoblastoma protein (Rb) is considered a promising biomarker.

Conclusion CDK4/6 inhibition might represent a substantial advance for patients with hormone receptor-positive HER2-negative BC. Results must be confirmed in phase III trials before any firm conclusions can be made regarding the future influence of CDK4/6 inhibition. There is an urgent need for prospective biomarker-driven trials to identify patients for whom CDK4/6 inhibition is cost-effective.

INTRODUCTION

Breast cancer (BC) is the most common cancer in women in almost all countries.\(^1\) Most often the disease is considered local at the time of diagnosis but eventually approximately 20% of patients will experience recurrence either as locoregional or distant disease.\(^2\) Despite the advances that have taken place in the past decade metastatic disease essentially remains incurable. The biological heterogeneity of the disease and development of resistance are regarded as major obstacles for obtaining more efficacious treatment approaches.

A hallmark of cancer is unrestrained growth due to overexpression of growth signals and loss of cell cycle checkpoint control.\(^3,4\) The retinoblastoma protein (Rb) represents a checkpoint regulator in mammalian cells. In its hypophosphorylated state Rb suppresses the expression of proteins that are essential for commitment to S phase and progression through the cell cycle. Normally, this is tightly regulated, but in malignancy, this transition point can become less closely regulated allowing for less controlled proliferation. The G1 cyclin-dependent kinases 4 and 6 (CDK 4 and 6) which function in complexes with the D-type cyclins (collectively named cyclin D) initiate the phosphorylation of Rb and override the repressive effects of Rb on cell cycle progression.\(^5,6\) Thus, the cyclin D-CDK4/6 complex is a key regulator of the Rb protein.

Often BC has aberrations throughout the cyclin-CDK (cyclin-dependent kinase)-retinoblastoma (Rb) pathway. Particularly, cyclin D1 (encoded by \textit{CCND1}) plays a crucial role in development of the disease. \textit{CCND1} has been found to be amplified in 15%–20% and cyclin D1 was overexpressed in up to 50% of all BC cases.\(^8\)

The possibility of using biological agents which target this basic cell cycle regulatory mechanism has come into great focus. First-generation CDK inhibitors tended to be less specific, targeting other CDKs in a broad fashion and were associated with chemother-apy-like toxicities and unacceptable safety profiles.\(^9,10\) More recently, a new generation of very specific CDK 4/6 inhibitors have been developed. At the moment, three CDK4/6 inhibitors have been tested in clinical BC trials: palbociclib (Ibrance, PD0332991; Pfizer, New York City, New York, USA), abemaciclib (LY2835219; Lilly, Indianapolis, Indiana, USA) and ribociclib (LEE011; Novartis, Basel, Switzerland). This review
investigates the efficacy and toxicity of specific CDK 4/6 inhibition in the treatment of BC.

METHODS
Articles included in this review were obtained by searching PubMed (1966–2016), EMBASE (1980–2016) and meeting abstracts from American Society of Clinical Oncology (ASCO) (2013–2016) and San Antonio Breast Cancer Symposium (2013–2016). The following searches were performed by two authors (DN and AP): ‘PD 0332991 OR palbociclib’ AND breast cancer (PUBMED: 67; EMBASE: 221), ‘LY2835219 OR abemaciclib’ AND breast cancer (PUBMED: 11; EMBASE: 43) and ‘ribociclib OR LEE011’ AND breast cancer (PUBMED: 10; EMBASE: 41). Titles and relevant abstracts were read. The following inclusion criteria were applied: clinical phase I, II or III trials excluding trials with a mixed tumour population in which data from patients with BC were not presented separately. Abstracts only reporting data on trial design were excluded. References for the selected articles were checked for additional relevant information.

ClinicalTrials.gov and EU Clinical Trial Register were searched for information about ongoing clinical trials using the above mentioned keywords. All searches were last updated June 2016. In order to avoid confusion regarding nomenclature we have chosen to designate the drugs palbociclib, abemaciclib and ribociclib throughout this review, irrespective of the name used in the original paper or abstract.

RESULTS
Trials in the preoperative or adjuvant setting
Only preliminary data from two phase II studies of palbociclib in the preoperative setting have been reported (table 1). An ongoing study of palbociclib in combination with letrozole for 4 months in 11 patients with oestrogen receptor (OR)-positive, HER2-negative BC and a tumour >2 cm showed an overall response rate (RR) of 89% and a pathological complete response (pCR) rate of 11%. A phase II trial of palbociclib plus anastrozole (+ goserelin in premenopausal patients) in a sequential design included 50 patients with stage 2 or 3 OR-positive HER2-negative BC. Of 40 evaluable patients 85% meet the primary end point, complete cell cycle arrest.

Three trials including patients with hormone receptor (HR)-positive, HER2-negative BC are currently ongoing in the adjuvant setting (table 2). A phase II study of palbociclib in combination with an aromatase inhibitor (AI) or tamoxifen plans to include 160 patients with stage II or III BC. The phase III PENELope-B study (NCT18644746) investigates 13 cycles of palbociclib or placebo in combination with standard endocrine therapy (ET) in patients with residual invasive disease after neoadjuvant chemotherapy. Finally, the phase III PALLAS trial (NCT02513394) investigates the addition of 2 years of palbociclib to standard ET in patients with stage II or stage III BC. Total planned accrual for this trial is 4600 patients with results expected in 2025. The trial includes a range of translation objectives and might be important for future selection of patients.

In the preoperative setting, we identified six ongoing phase II trials investigating palbociclib, one investigating abemaciclib and one investigating ribociclib in HR-positive, HER2-negative BC; seven of the trials are randomised (table 2).

Trials in advanced BC
Palbociclib
Phase I
Twelve postmenopausal women with OR-positive, HER2-negative metastatic (M)BC were enrolled in a phase I study investigating the safety and tolerability of palbociclib plus letrozole for first-line treatment (table 3). No drug-drug interactions were observed. Three patients (25%) experienced a partial response (PR) and nine patients (75%) experienced tumour stabilisation. Most important dose limiting toxicity (DLT) was grade 4 neutropenia. Besides neutropenia common adverse events (AEs) were leucopenia and fatigue (table 4).

| Reference | Therapy | Phase | Patient characteristics | Number of patients | Response rate (%) | Grade 3/4 toxicity |
|-----------|---------|-------|-------------------------|--------------------|------------------|-------------------|
| Chow et al | Palbociclib + letrozole (4 months preoperatively) | II, OOTR-N007 | OR+, HER2-postmenopausal tumour >2 cm, not T3 N1, T4 or N 2,3 | 11 (9 completed); of 45 planned; the study is ongoing | pCR 11% PR 78%, RR 89% | 44% neutropenia |
| Ma et al | Anastrozole + goserelin (if premenopausal) + palbociclib | II | OR+, HER2-stage 2 or 3 | 50 (40 evaluable) | Complete cell cycle arrest 85% | NR |

HER2, human epidermal receptor 2; NR, not reported; OR, oestrogen receptor; ORR, overall response rate; pCR, pathological complete response; PR, partial response; RR, response rate.
| Clinical trial.gov identifier | Therapy | Phase | Patient characteristics | Number of patients | Primary end points | Estimated study completion |
|-------------------------------|---------|-------|------------------------|--------------------|--------------------|--------------------------|
| Adjuvant                      |         |       |                        |                    |                    |                          |
| NCT02040857                  | Palbociclib + AI or tamoxifen | II    | HR+, HER2- stage 2 or 3 (+ men) | 160                | Treatment discontinuation rate | June 2019, recruiting |
| NCT18644746                  | Palbociclib (13 cycles) + Standard ET Placebo + Standard ET | III, PENELOPE-B | HR+, HER2- Residual invasive disease after neoadjuvant chemotherapy; adequate surgery High CPS-EG score | 1100               | Invasive DFS | November 2023, recruiting |
| NCT02513994                  | Palbociclib 2 years + standard ET Standard ET | III, PALLAS | HR+, HER2- Stage 2 or 3 (+men) | 4600               | Invasive DFS | September 2025, recruiting |
| Presurgical                  |         |       |                        |                    |                    |                          |
| NCT01709370                  | Palbociclib + letrozole (16 weeks) | II    | OR+, HER2- Postmenopausal tumour>2cm No1T3N1, T4, N2 or N3 | 45                 | RR | NR, study status last verified October 2012 |
| NCT01723774                  | Anastrozole + goserelin (if premenopausal) + palbociclib | II    | OR+, HER2- stage 2 or 3 | 29                 | Complete cell cycle arrest in women without PIK3CA hot spot mutation | February 2016, recruiting |
| NCT02296801                  | Letrozole → palbociclib + letrozole Palbociclib → palbociclib + letrozole Palbociclib + letrozole 14 weeks | II, PALLET neo-adjuvant | OR+, HER2-postmenopausal operable, tumour>2cm | 306                | Proliferation (Ki67) | January 2015, recruiting |
| NCT02400567                  | FEC → docetaxel palbociclib + letrozole | II, NeoPAL Randomised, | Luminal A + nodal involvement or luminal B postmenopausal stage – 2-3A | 132                | Number with residual tumour in breast or lymph node | April 2019, recruiting |
| Eudract number 2014-000809-12 | Palbociclib + standard ET standard ET | II, PREDIXLumA (part of a translational study based of molecular subtypes) | Luminal A >2cm, no lymph node metastases | 200 (whole trial) | pCR | NR, recruiting |
| Eudract number 2014-000810-12 | Palbociclib + standard ET standard ET | II, PREDIXLumB (part of a translational study based of molecular subtypes) | Luminal B>2cm and/or lymph node metastases | 200 (whole trial) | pCR | NR, recruiting |
| NCT02008734                  | Control palbociclib (125 mg/day for 14 days) Palbociclib (100 mg/d for 21 days) | II, POP Randomised (3:1) | Untreated, operable early BC (>15 mm) Not candidate for neoadjuvant chemotherapy | 105                | Antiproliferative response | January 2016, recruiting |
| Abemaciclib                  |         |       |                        |                    |                    |                          |
| NCT02441946                  | Abemaciclib + loperamide 2 weeks Abemaciclib + loperamide + anastrozole 2 weeks Anastrozole 2 weeks Followed by 14 weeks abemaciclib + anastrozole + loperamide | II, NeoMONARCH | ER+, HER2- Postmenopausal tumour>1cm, ET deemed suitable | 220                | Ki67 expression at 12 weeks | February 2017, recruiting |
| Ribociclib                   |         |       |                        |                    |                    |                          |
| NCT01919229                  | Ribociclib (400 mg) + letrozole Ribociclib (600 mg) + letrozole Letrozole | II, MONALEESA-1 | HR+, HER2- Postmenopausal, tumour>1.0 cm | 14                 | Cell cycle response rate | Completed, no results published |

AI, aromatase inhibitor; BC, breast cancer; CPS-OEG, clinical-pathological stage-oestrogen/grade score; ET, endocrine therapy; HR, hormone receptor; NR, not reported; pCR, pathological complete response; RR response rate; DFS, disease free survival.
| Reference | Therapy | Phase | Patient characteristics | Number of patients | Response rate | Median PFS | Median OS |
|-----------|---------|-------|-------------------------|--------------------|--------------|------------|-----------|
| Slamon et al | Palbociclib + letrozole vs letrozole | I | HR+, HER2-postmenopausal MBC first line | 12 | PR 25% | 3.7 (1.9–5.1) | NR |
| Clark et al | Palbociclib + paclitaxel | I | 84% HR, HER2-; 5% OR/HER2+; 11% HR+; HER2- MBC; 76% ≥2lines of hormonal therapy; 76% ≥2lines of chemotherapy | 37 | PR 5% | 3.7 (1.9–5.1) | NR |
| DeMichele et al | Palbociclib | Phase II | 13 | PR 25% | 3.7 (1.9–5.1) | NR |
| Finn et al | Palbociclib | Phase II | OR+, HER2-postmenopausal ABC | Part 1: 66 | RR 42.1% | 24.8% | Data immature |
| Finn et al | Palbociclib + letrozole | Phase II | OR+, HER2-postmenopausal ABC | Part 2: 99 | RR 42.1% | 24.8% | Data immature |
| Turner et al, Cristofanilli et al | Abemaciclib + fulvestrant | III, PALOMA-3 (2:1) | HR+, HER2- | 347 | 19% (95% CI 15.0 to 23.6) | 9.5 (2.0–11.0) | NR |
| Tolaney et al | Abemaciclib + (A) letrozole, (B) anastrozole (C) tamoxifen (D) exemestane (E) exemestane + trastuzumab | I | HR+, HER2- (A–E) or HER2+ (F) | 65 | AB (63 pts); DOR (duration NR): 67% C (16 pts): 75% | 14.8% | 14.8% |
| Patnaik et al | Abemaciclib + fulvestrant | Phase I, 2 cohorts | MBC | 47 | HR+: PR 36% (25% confirmed) | 4.6 (3.5–5.6) | NR |
| Dickler et al | Abemaciclib | Phase II | HR+, HER2- MBC | 132 | RR (confirmed) 17.4% | 5.7 | 9.5 (2.0–11.0) | NR |

**Table 3: Efficacy of CDK4/6 inhibitors in the metastatic setting**

ABC, advanced breast cancer; Adj, adjuvant; CBR, clinical benefit rate; CR, complete response; ET, endocrine therapy; OR, oestrogen receptor; OS, overall survival; HR, hormone receptor; NR, not reported; NSAI, non-steroidal aromatase inhibitor; ORR, overall response rate; pts, patients; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.
| Reference | Therapy | Neutropenia (%) | Febrile neutropenia (%) | Leucopenia (%) | Anaemia (%) | Thrombocytopenia (%) | Fatigue (%) | Other (%) | Discontinuation (%) |
|-----------|---------|-----------------|------------------------|---------------|-------------|---------------------|------------|----------|---------------------|
| Slamon et al<sup>13</sup> | Letrozole + palbociclib vs letrozole | 17 (grade 4) (~2 DLT) | | | | | | | 8 (dose interruption) |
| Clark et al<sup>14</sup> | Palbociclib + paclitaxel | 59 | 4 (DLT) | - | - | - | - | Grade 3 AST/ALT(DLT) | 67 (dose interruption) |
| DeMichele et al<sup>15</sup> | Palbociclib | 51 | 3 | 51 | 5 | 22 | 0 | Lymphopenia 30 | 3 |
| Finn et al<sup>16,17</sup> | Letrozole + palbociclib | All grades 79.5; grade 3: 56.1 | 51 | 0 | 19 | 6 | 2 | 4 | 13 |
| Finn et al<sup>17</sup> | Letrozole | All grades 6.3 | 1 | 0 | 0 | 1 | 0 | 1 | 2 |
| Turner et al<sup>18</sup>, Cristofanilli et al<sup>19</sup> | Palbociclib + fulvestrant | 65. | 1 | 28 | 3 | 2 | 2 | - | 4 |
| | Placebo + fulvestrant + goserelin | 1 | 1 | 2 | 2 | 0 | 1 | | 2 |
| Tolaney et al<sup>20</sup> | Abemaciclib + (A) letrozole (B) anastrozole (C) tamoxifen (D) exemestane (E) exemestane + everolimus (F) exemestane + trastuzumab | 17 | | | | | | | 14 |
| | Abemaciclib | | | | | | | | 8 |
| Patnaik et al<sup>21</sup> | Abemaciclib* | 11 | 23 | | | | | | 2 |
| | Abemaciclib + fulvestrant | 31 | | | | | | | 8 |
| Dickler et al<sup>22</sup> | Abemaciclib | | | | | | | | 4.8 |
| Juric et al<sup>23,24</sup> | A1: ribociclib + letrozole. A2: alpelisib + letrozole | 43 (A1) | 2 (A1) | | | | | | 11 (A3) |
| | A3: ribociclib + letrozole + BYL719 | 22 (A3) | | | | | | | |
| Bardia et al<sup>25,26</sup> | Ribociclib + everolimus + exemestane | 45.7 | 8.6 | 5.7 | | | | | 6 DLT: 1 febrile neutropenia, 2 ALT elevations, 2 thrombocytopenia, 1 mucositis |
| | Ribociclib + exemestane | | | | | | | | |

*Toxicity reported for 132 patients (47 with breast cancer).

AE, adverse event; ALT, alanine transaminase; AST, aspartate transaminase; DLT, dose limiting toxicity; NR, not reported.
A phase I trial evaluated the combination of palbociclib and paclitaxel in Rb-expressing advanced (A) BC. In the dose escalating part of the study a 5 days schedule for palbociclib was used. Due to frequent neutropenia grade 3/4 this schedule was changed to a 3 days treatment in the dose expansion part. The study demonstrated 41% PRs and 30% stable disease (SD) (tables 3 and 4).\textsuperscript{14}

**Phase II**

A phase II trial of palbociclib monotherapy in heavily pretreated women with Rb-positive MBC included 37 patients among which 84% had HR-positive, HER2-negative disease.\textsuperscript{15} Overall, median progression-free survival (PFS) was 3.7 months. Median PFS for patients with HR-positive, HER2-negative disease was 5.1 months. Patients with HR-positive BC had significantly longer PFS compared with the HR-negative group (4.5 months vs 1.5 months, p=0.03). Five per cent had PR and 19% clinical benefit rate (CBR) (CR + PR + SD ≥6months) in the HR-negative cohort. In the HR-positive cohort the figures were 6% and 21%, respectively. Grade 3/4 toxicities were transient neutropenia (51%) and thrombocytopenia (22%). One episode of neutropenic sepsis (3%) was registered. Twenty-four per cent of the patients had treatment interruption and 51% had dose reductions due to cytopenia. One patient (3%) discontinued treatment due to toxicity (fatigue) (table 4). No biomarkers (Rb expression/localisation, Ki-67, p16 loss or CCND1 amplification) were identified.

In the randomised phase II study PALAMO-1/TRIO-18 (NCT00721409) the effect of palbociclib plus letrozole versus letrozole alone for first-line treatment of OR-positive, HER2-negative ABC was investigated.\textsuperscript{16} The study was designed as a two-part study. The first part enrolled patients not previously treated for ABC. In the second part the patients were additionally screened for CCND1 amplification and/or loss of p16.\textsuperscript{16} Sixty-six and 99 patients were randomised in the two parts of the study, respectively. For all patients, the RR and CBR (SD ≥24 weeks) for the letrozole + palbociclib arm (n=84) were 42% and 80%, respectively, and 32% and 57% in the letrozole monotherapy arm (n=81) (p=0.0046). Both parts of the study showed significantly increased PFS (table 3). PFS for all patients (n=165) was 20.2 months compared with 10.2 months in the letrozole arm with an HR of 0.488 (95%CI: 0.319 to 0.748; p=0.0004). However, the overall survival (OS) was not significantly different in the two arms. The effect of the combination was consistent across demographic subgroups. The most common treatment-related grade 3–4 AEs in the combination arm were neutropenia (54% vs 1%), leukopenia (19% vs 0%), anaemia (6% vs 1%) and fatigue (4% vs 1%). No cases of febrile neutropenia were reported (table 4). Totally, 13% of patients in the palbociclib arm and 2% in the letrozole monotherapy arm discontinued treatment due to AEs. Long-term safety results suggested no evidence of cumulative toxicity or late onset of toxicity.\textsuperscript{15}

None of the measured genetic changes either alone or in combination could be used for selection of patients.

**Phase III**

Recently, preliminary results from the phase III double-blind PALOMA-2 study evaluating first-line letrozole +/- palbociclib in 666 HR-positive, HER2-negative patients with ABC were published. In both groups, 33% of patients had de novo advanced disease and 43% had not received prior ET. The study confirmed results from PALAMO-1 (NCT00721409) with a median PFS in the palbociclib arm of 28.4 months versus 14.5 months in the letrozole monotherapy arm (HR 0.58; CI 0.46 to 0.72, p<0.000001), whereas RR was 42.1% and 34.7% (p=0.051), respectively. OS data are immature. The most common grade 3 AE in the palbociclib group was neutropenia (56.1%); febrile neutropenia was seen in 2.5% of patients.\textsuperscript{17}

The double blind phase III PALOMO-3 study (NCT01942135) compared palbociclib plus fulvestrant versus fulvestrant plus placebo in 521 HR-positive, HER2-negative patients with ABC whose cancer had relapsed or progressed on prior ET.\textsuperscript{18,19} Premenopausal and perimenopausal women also received goserelin. A total of 79% of the patients was considered sensitive to ET and approximately a third of the patients had received chemotherapy for ABC. Approximately 40% had previously received an aromatase inhibitor (AI), 15% tamoxifen and 45% both drugs.\textsuperscript{18} Median PFS was 9.5 months in the palbociclib plus fulvestrant group versus 4.6 months in the fulvestrant plus placebo arm (HR 0.46; 95% CI 0.36 to 0.59, p<0.0001).\textsuperscript{19} In addition, global quality of life was generally maintained with palbociclib but deteriorated in the placebo arm. The benefit from palbociclib was seen in both premenopausal and postmenopausal women. Side effect grade 3 and grade 4 included neutropenia (65.0% vs 1.0%), leucopenia (28% vs 1%), anaemia (3% vs 2%), thrombocytopenia (3% vs 0%) and fatigue (2% vs 1%) in the palbociclib and monotherapy arms, respectively.\textsuperscript{18} Febrile neutropenia was reported in 1% in both arms. The rate of discontinuation was 4% and 2% with palbociclib and placebo, respectively (tables 3 and 4). The median PFS observed in the placebo + fulvestrant arm was inferior to that in prior studies of ET alone.\textsuperscript{20} This could probably be explained by a younger and more heavily treated study population.

**Abemaciclib**

**Phase I**

Preliminary data from a phase I study of abemaciclib in patients with five different tumour types (n=132) have been presented.\textsuperscript{21} The MBC cohort included 47 patients (36 HR-positive) with a median of seven prior systemic therapies. Nineteen per cent of these patients obtained PR, and 51% experienced SD (36% >24 weeks). Disease control (CR + PR + SD) rate was 70% for all patients and 81% for HR-positive patients. The median PFS was 5.8 months for all patients and 9.1 months for HR-positive
patients. Most common treatment-related grade 3 or grade 4 AEs in the expansion cohorts (n=132) were diarrhoea (5%), nausea (3%), fatigue (2%), vomiting (2%) and neutropenia (11%). No febrile neutropenia was reported. The phase I study was expanded to evaluate the efficacy of abemaciclib plus fulvestrant in HR-positive MBC. The patients had a median of four prior systemic therapies. Preliminary results reported 62% confirmed and 23% unconfirmed PRs. Observed grade 3 AEs were diarrhoea (8%), fatigue (8%), neutropenia (31%) and leucopenia (23%). No grade 4 events were reported.

A phase I study of abemaciclib in combination with different ETs for BC demonstrated disease control rates of 67% and 75% for patients who received abemaciclib in combination with non-steroid (NS)AI and tamoxifen, respectively. The most common grade 3 toxicities were diarrhoea 31%, neutropenia 17%, fatigue 14% and nausea 6%. No grade 4 events were reported.

**Phase II**

The phase II MONARCH-1 study (NCT02102490) evaluated abemaciclib monotherapy in 132 patients with HR-positive, HER2-negative MBCs who had previously received one to two lines of chemotherapy. Preliminary data showed an RR of 17.4% and a CBR of 42.4% with a median PFS of 5.7 months. The most common AEs (grade) were diarrhoea, fatigue, decreased appetite and abdominal pain. Totally 6.8% of the patients discontinued treatment due to toxicity.

**Ribociclib**

**Phase I**

A phase Ib study of ribociclib and alpelisib (BYL719, α-specific PIK3 inhibitor) in combination with letrozole in postmenopausal women with OR-positive, HER2-negative MBC was designed with three arms, in which patients received the following: A1: ribociclib + letrozole, A2: alpelisib + letrozole and in A3: ribociclib + alpelisib + letrozole. Preliminary data from cohorts A1 and A3 have been presented. At the time of presentation, 47 patients were enrolled in A1. An RR of 5% and a CBRCRB of 32% were demonstrated in 19 patients who had received previous treatment; whereas RR was 39% and CBR was 75% among 28 treatment-naïve patients. Neutropenia grade 3 or grade 4, lymphopenia and leucopenia were reported in 43%, 4% and 2% of the patients, respectively. Furthermore, hyperglycaemia grade 3–4 was seen in 14% of the patients. Totally, 36 patients were enrolled in A3. Among 27 evaluable patients 7% had PR and 15% an unconfirmed PR. The most frequent grade 3–4 AEs were neutropenia (22%), hyperglycaemia (14%), fatigue (11%) and nausea (6%).

More recently, preliminary data from a two-armed study investigating the effect of combining ribociclib, everolimus and exemestane in postmenopausal women with NSAI-resistant ABC have been presented. In the first arm patients received escalating doses of ribociclib, everolimus and exemestane. In the second arm patients received a fixed dose of ribociclib and exemestane. At the time of presentation, 84 patients were included, 70 patients received the triplet combination. Results for the doublet arm have not been presented. Six patients experienced DLTs. Observed AEs were mainly haematological, most common were neutropenia and leucopenia. Complete response (CR) was reported in 1.8%, PR in 9.1% (3.6% confirmed) and disease control defined as CR + PR + SD + non-CR non-progressive disease (PD) in 70.9%.

**Ongoing trials in the advanced/metastatic setting**

Most identified trials are performed in patients with OR-positive, HER2-negative BC (table 5). Totally, seven trials investigate palbociclib. Two phase III trials evaluate palbociclib in combination with ET. The PEARL trial (NCT02028507) compares palbociclib + exemestane with capcitabine, while two trials evaluate palbociclib in combination with HER2-targeted therapy in HER2-positive BC.

Five studies evaluate abemaciclib in combination with different ETs and/or, everolimus and/or trastuzumab. One phase II trial evaluates the drug alone or in combination with ETand/or trastuzumab in patients with brain metastases. Finally, ribociclib is investigated in eight trials, of which five investigate ribociclib in combination with ET, everolimus or a PIK3 inhibitor.

**DISCUSSION**

Three oral agents selectively targeting CDK4/6 are currently in development. The chemical structures of palbociclib and ribociclib are very similar whereas the structure of abemaciclib is different. In general, however, the mechanisms of action of the agents are presumably identical and preclinical anticancer activities have appeared to be qualitatively similar. An important difference between the three CDK4/6 inhibitors seems to be, that abemaciclib has shown a more potent ability to cross the blood-brain barrier making it a potential agent to treat brain metastases. In contrast, using an orthotopic brain tumour model Parrish et al29 have demonstrated limited brain distribution and efficacy of palbociclib. A phase II study of abemaciclib ± ET/trastuzumab in patients with BC and brain metastases is ongoing.

**Specific CDK4/6 inhibition in BC subtypes**

**OR-positive HER2-negative disease**

Not surprising given the different drivers of the molecular subtypes in BC and their differences in Rb pathway alterations, the sensitivity to CDK4/6 inhibition differed. In oestrogen-driven BC oncogenic signalling through oestrogen stimulated the cyclin D-CDK4/6-dependent phosphorylation of Rb, and this proliferative stimulus was augmented by amplification of CCND1 or loss of expression of the cyclin D-CDK4/6 inhibitor p16. This suggests that especially OR-positive tumours could be vulnerable.
Table 5  Ongoing trials with CDK 4/6 inhibitors in ABC

| Clinical trial.gov identifier | Therapy | Phase | Patient characteristics | Number of patients | Line of therapy | Chemotherapy for MBC | Primary end points | Estimated study completion |
|------------------------------|---------|-------|-------------------------|--------------------|-----------------|----------------------|--------------------|--------------------------|
| NCT01684215                 | Palbociclib | I/II  | OR+, HER2-Japanese, phase I: solid tumours | 138                | DLT             | None                 | 1-year PFS         | January 2017, recruiting |
| NCT01976169                 | Palbociclib + trastuzumab-DM1 (‘DM1’) | I     | HER2+; ABC, prior trastuzumab Rb proficient (Rb normal and low p16) | 17                 | No criteria       | No criteria          | DLT                | August 2015, recruiting  |
| NCT023844239                | Palbociclib (100 mg) + fulvestrant or tamoxifen | II    | HER2-postmenopausal, MBC or LABC ≤1 line of HER2-directed therapy | 70                 | ≤2 prior lines    | PD (16 weeks)        | August 2017 (not initiated March 2015) |
| NCT02444640                 | Palbociclib + letrozole | II    | Asian, OR+, HER2- postmenopausal, ≥12 months from adjuvant NSA | 138                | No criteria       | No criteria          | 6 months          | December 2019             |
| NCT022997438                | Palbociclib + letrozole | III   | PALAMO-4                 | 330                | First line       | None                 | PFS                | October 2017, recruiting  |
| NCT02028607                 | Palbociclib + exemestane | III   | EVER-BIRAD-2             | 348                | First or Second line | ≤1                 | PFS                | January 2018             |
| NCT02333370                 | Ribociclib + letrozole | Ib/II | OR+, HER2-Postmenopausal ABC | 112                | First line       | None                 | PFS (phase II)     | February 2021, recruiting |
| NCT01857193                 | Ribociclib + everolimus + exemestane | Ib/II | OR+, HER2-postmenopausal, LABC or MBC, ad NSA | 185                | ≤1               | First line           | Phase Ib: DLT Phase III: PFS | May 2016, recruiting |
| NCT01872260                 | Ribociclib + letrozole | II    | BLY219 (PI3Kα inhibitor) + letrozole Everolimus + exemestane | 300                | Dose escalation: first line | Dose escalation: first line | Phase Ib: DLT Phase III: PFS | May 2017, recruiting |
| NCT02088644                 | Ribociclib + fulvestrant | Ib/II | HR+, HER2-Postmenopausal, LABC or MBC, ad NSA | 216                | Dose escalation: first line | Dose escalation: first line | Phase Ib: DLT Phase III: PFS | February 2019, recruiting |
| NCT01958021                 | Ribociclib + letrozole | III   | MONALEESA-2              | 650                | First line       | None                 | PFS                | August 2017, recruiting  |
| NCT02422515                 | Ribociclib + fulvestrant | III   | MONALEESA-3              | 660                | First or second line | None              | PFS                | May 2020, not yet open    |
| NCT02278120                 | Ribociclib + anastrozole/tamoxifen + goserelin | III   | MONALEESA-7              | 660                | First line       | None                 | PFS                | February 2018, recruiting |
| NCT02107073                 | Abemaciclib + fulvestrant | III   | MONARCH 2                | 630                | First or Second line | None              | PFS                | February 2020, recruiting |
| NCT02244621                 | Abemaciclib + tamoxifen | III   | MONARCH 3                | 450                | First line       | None                 | PFS                | July 2021, recruiting  |
| NCT0257133                  | Abemaciclib + letrozole | I     | MBC, HR+, HER2- or HER2+ (trastuzumab) Combination | 102                | ≥1               | Combination          | Number with drug-related AE | November 2016, recruiting |
| NCT02050210                 | Abemaciclib + ET + trastuzumab | II    | HR+, brain metastases | 120                | No criteria       | No criteria          | Intracranial RR     | NR, ongoing            |

ABC, advanced breast cancer; AE, adverse event; ET, endocrine therapy; OR, oestrogen receptor; HR, hormone receptor; LABC, locally advanced breast cancer; MBC, metastatic breast cancer; NR, not reported; NSAI, non-steroidal aromatase inhibitor; PD, progressive disease, PFS, progression-free survival; RR, response rate.
for drug-induced CDK4/6 inhibition. Preclinical studies have shown optimal activity of CDK 4/6 inhibitors in OR-positive, HER-2 negative BC and synergistic effect with tamoxifen, fulvestrant and letrozole.24-28 Furthermore, studies of endocrine-resistant cell lines/subpopulations have shown cell cycle arrest and suppression of cell proliferation after addition of palbociclib suggesting that the compound could be effective in OR-resistant disease.30

Palbociclib received a granted accelerated approval from the Food and Drug Administration (FDA) in February 2015 for use in combination with letrozole based on data from the randomised phase 2 PALAMO-1 trial (NCT00721409) and in February 2016 for use in combination with fulvestrant based on the PALAMO-3 trial (NCT01942135) which was stopped early based on efficacy seen in the interim analysis.39, 36 Median PFS was 9.2 months for palbociclib plus fulvestrant and 3.8 months for placebo plus fulvestrant (HR=0.422; p<0.000001).19 More recently, preliminary results from a phase III randomised, double-blinded study evaluating letrozole and palbociclib versus letrozole as first line treatment of women with HR+, HER2– MBC (PALAMO-2; NCT01740427) have been published confirming results from PALOMA-1. This study showed that addition of palbociclib to letrozole increased PFS by 10 months.

So far, data except for PALAMO-2 (NCT01740427) and PALAMO-3 (NCT01942135) are obtained by phase I and II studies. The clinical studies are primarily presented in abstract forms. Thus, results need to be confirmed before any conclusions can be made. Several phase II and phase III studies have estimated study completions in 2015/2016.

A major limitation of ET is intrinsic and acquired resistance. Although expression of OR is strongly predictive of response to ETs, approximately a third of OR-positive BCs do not respond or relapse after an initial response.57 The PALAMO-3 study (NCT01942135) suggests that palbociclib has activity in patients with endocrine-resistant disease and it is suggested that targeting CDK4/6 may represent a therapeutic strategy across diverse mechanisms of resistance.19 On the other hand, OR-positive BC is biologically heterogeneous and many patients have long-lasting benefit of endocrine monotherapy.32-38

Lately, palbociclib is given as an option for treatment of OR-positive HER2-negative ABC in both ASCO and NCCN guidelines.59, 60

HER2-positive disease
A synergistic effect was seen in a preclinical study when treating HER2-positive cell lines with trastuzumab and palbociclib simultaneously. Preclinical studies have also demonstrated profound cytostatic arrest, induction of senescence and inhibition of invasive properties in HER2-positive cell culture models after addition of palbociclib.41 The drug significantly suppressed Ki67 in HER2-positive BC mouse models and human primary tumour explants.62 Additionally, in models of acquired resistance to HER2-targeting therapies palbociclib blocked proliferation and seemed to act synergistically with trastuzumab and T-DM1.33, 45, 44 Yet no clinical studies have been published. A few studies are ongoing combining palbociclib or abemaciclib with HER2-targeted therapy most often in combination ET in OR-positive HER2-positive BC (NCT01976169, NCT02448420, NCT0205713, Eudract 2014-004010-28).

Triple-negative disease
It has been debated whether the CDK4/6 inhibitors can be used in co-treatment with a chemotherapeutic agent, as most chemotherapeutic agents act specifically on proliferating cells. A preclinical study in triple-negative BC demonstrated an additive cytostatic effect between palbociclib and doxorubicin, but it appeared that palbociclib inhibited doxorubicin-mediated cell death signalling.45 Studies of the long-term effect of combined therapy indicated that palbociclib maintained viability of Rb-proficient cells and thereby could result in tumour cell outgrowth following doxorubicin treatment.45 Furthermore, co-administration of palbociclib and paclitaxel reduced the cytotoxicity of this chemotherapeutic. Importantly, subsequent experiments demonstrated that synchronisation with CDK4/6 inhibitors improved the cytotoxicity of doxorubicin as well as paclitaxel, highlighting the importance of timing when using combination therapy.45 In contrast, treatment with the cytotoxic agent gemcitabine in combination with abemaciclib in preclinical studies seemed to induce a greater inhibition of tumour growth than either treatment alone.15

Preliminary results from a phase I study of palbociclib and paclitaxel in Rb-expressing advanced BC among whom approximately 50% had received prior taxane demonstrated 41% PRs and 30% stable disease (SD).44 The efficacy was comparable to results obtained from a phase II study of weekly paclitaxel in a similar group of patients showing an RR of 22% and an SD of 42%.48

Safety
In general, the toxicity of the inhibitors has been favourable. The toxicity of all three agents has been predominantly haematological characterised by limited neutropenia, which was expected from the mechanism of action and were considered as on-target, antiproliferative responses. For palbociclib, the haematological AEs acted in general in a non-cumulative manner, were reversible, short lasting with lack of clinical morbidity and pancytopenia.49 Despite the high rate of neutropenia only few cases of neutropenic fever were recorded. Other common AEs were infections, fatigue and gastrointestinal toxicity. For ribociclib a relative high rate of grade 3 hyperglycaemia has been reported.50 Yet data are too limited to differentiate between toxicity profiles of the compounds.

Potential predictive biomarkers
Preclinical studies have shown that the effect of CDK4/6 inhibitors was dependent on an intact, functional Rb
protein. Loss of Rb expression has been found to occur in 20–30% of BCs. However, the incidence of Rb loss was dependent on the clinical subtype and was more common in triple-negative BC compared with other subtypes. More than 90% of OR-positive BCs have been found to express a functional Rb protein. As expected from the extensive but incomplete overlap between clinical and intrinsic subtypes, Rb pathway alterations also differed by molecular subtypes. Thus, luminal A tumours were more likely to have an intact Rb pathway than the other subtypes. Basal-like tumours had—as expected—from the overlap with clinical triple negative cancer—often Rb loss. While the majority of BCs maintained functioning Rb, the CDK4/6-cyclin D pathway may be disrupted by a number of other mechanisms, for example, CCND1 amplification or overexpression of cyclin D1. Especially, CCND1 amplification was frequent in luminal tumours, albeit most notably in luminal B.

Particular attention has been paid to the search for potential biomarkers for efficacy of CDK 4/6 inhibitors. Increased expression of cyclin D and Rb protein was associated with response in vitro, as was decreased expression of p16. Preclinical studies with palbociclib and abemaciclib concluded that only Rb-proficient cells responded to treatment with these agents and that cell lines most sensitive to CDK4/6 inhibition had increased expression of RB1, CCND1 and a decreased expression of CDKN2A (p16). However, results from the same studies illustrated that Rb expression alone was not a guarantee of response to palbociclib, as some basal cell lines with Rb present were resistant to palbociclib treatment. Furthermore, results from a phase II study of single-agent palbociclib indicated that BC cells more likely responded to treatment if they expressed high Rb nuclear levels, low Ki67 indices and/or loss of p16, whereas CCND1 status did not seem to predict a response. On the other hand, results from the PALOMA-1 trial indicated that Ki67, CCND1 and CDKN2A expression did not influence the efficacy of treatment in relation to PFS.

Two ongoing studies (a phase I study with T-DM1 and palbociclib (NCT01976160) and a phase I study with paclitaxel and palbociclib (NCT01320592)) have Rb expression as one of their inclusion criteria. Thus, for the present Rb status is the most promising biomarker. Additional clinical trials have to be conducted before conclusions can be made regarding useful biomarkers.

CONCLUSION

The specific CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib inhibit cell cycle progression in an Rb-dependent manner. A randomised phase II and a phase III trial of palbociclib plus ET versus ET have shown significantly increased PFS when compared with ET alone in first-line and second-line treatments for HR-positive HER2-negative ABC. At the moment several phase III studies are ongoing with all three CDK4/6 inhibitors. CDK4/6 inhibition might represent substantial advances for selected patients. However, there is an urgent need for prospective biomarker-driven trials to identify patients for whom these treatments are cost-effective.

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