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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.[5–7] 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 *CCDN1*) plays a crucial role in development of the disease. *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 chemotherapy-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%.[11] Manageable neutropenia was seen in 44% of the patients.[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.[12]

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).[13] 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).[13]

Table 1 Efficacy of CDK4/6 inhibitors in the preoperative setting

Reference	Therapy	Phase	Patient characteristics	Number of patients	Response rate (%)	Grade 3/4 toxicity
Chow *et al*[11]	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*[58]	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.

Table 2 Ongoing trials with preoperative or adjuvant CDK4/6 inhibitors in primary BC

Clinical trial.gov identifier	Therapy	Phase	Patient characteristics	Number of patients	Primary end points	Estimated study completion
Adjuvant						
Palbociclib						
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
NCT02513394	Palbociclib 2 years + standard ET Standard ET	III, PALLAS	HR+, HER2- Stage 2 or 3 (+men)	4600	Invasive DFS	September 2025, recruiting
Presurgical						
Palbociclib						
NCT01709370	Palbociclib + letrozole (16 weeks)	II	OR+, HER2- Postmenopausal tumour≥2 cm Not T3N1, 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 Letrozole → palbociclib + letrozole Palboclib → palbociclib +letrozole Palcociclib + letrozole 14 weeks	II, PALLET neoadjuvant	OR+, HER2-postmenopausal operable, tumour≥2 cm	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 >2 cm, 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>2 cm and/or lymph node metastases	200 (whole trial)	pCR	NR, recruiting
NCT02008734	Control palbociclib (125 m/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≥1 cm, ET deemed suitable	220	Ki67 expression at 2 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-OG, 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.

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

Reference	Therapy	Phase	Patient characteristics	Number of patients	Response rate	Median PFS months	Median OS months
Palbociclib							
Slamon *et al*[13]	Letrozole + palbociclib vs letrozole	I	HR+, HER2-postmenopausal MBC first line	12	PR 25% SD 75%		
Clark *et al*[14]	Palbociclib + paclitaxel	I	Rb-expression ABC (+men) 78% previous taxane	15+12 (dose expansion; new schedule)	PR 41% SD 30%	NR	
DeMichele *et al*[49]	Palbociclib	Phase II	84% HR+HER2- 5% OR+/HER2+11% HR-,HER2- MBC 65% ≥2 lines of hormonal therapy 76% ≥2 lines of chemotherapy	37	PR 5% CBR (SD≥6 months) 19%: HR+, HER2-: PR 6% CBR (SD≥6 months) 29%	3.7 (1.9–5.1) HR+/HER2-: 3.8 (1.9–5.8)	NR
Finn *et al*[16 36]	Letrozole + palbociclib Letrozole	Phase II PALOMA-1	OR+, HER2-postmenopausal ABC First line No adj therapy 52%/46% Adj TAM 29%/30% Adj AI 17%/17% Adj chemotherapy 40%/46% Part 2: + screened for *CCND1* amplification and/or loss of p16	Part 1:66 Part 2: 99	(N=84): RR 31% CBR 68% (N=81): RR 26% CBR 44%	Part 1: HR 0.299 (95% CI, 0.159 to 0.572; p=0.0001). Part 2: PFS: 26.2 vs 7.5 HR 0.032 (95% CI, 0.19 to 0.56; p<0.001). Part 2: PFS: 26.2 vs 7.5 HR 0.032 (95% CI, 0.19 to 0.56; p<0.001). Part 1+2 (N=165): PFS: 20.2 vs 10.2 HR 0.488 (95% CI, 0.319 to 0.74; p=0.0004).	(N=61): 37.5 vs 33.3.
Finn *et al*[17]	Palbociclib + letrozole Letrozole	III, PALOMA-2 (2:1)	OR+, HER2– Postmenopausal ABC first line Prior ET 57%	666	RR 42.1% 34.7% (p=0.031) CBR (not defined) 84.9 vs 70.3; p <0.0001	24.8% 14.8% HR 0.58 (95% CI 0.46 to 0.72; p<0.000001)	Data immature
Turner *et al*[59], Cristofanilli *et al*[18]	Palbociclib + fulvestrant Placebo + fulvestrant ± goselin	III, PALOMA-3 (2:1)	HR+, HER2– ABC Relapse or PD on prior ET Prior AI≈85% Prior TAM≈60% ≤1 line of chemotherapy	347 174	19 (95% CI15.0 to 23.6) 9 (4.9 13.8)(p=0.0019) CBR (CR+PR+SD≥24 weeks) 67 (61.3–71.5) 40 (32.3–47.3) (p >0.0001)	9.5 (2.0–11.0) 4.6 (3.5–5.6) (HR 0.46, 95% CI 0.36 to 0.59; p<0.0001)	NR
Abemaciclib							
Tolaney *et al*[23]	Abemaciclib + (A) letrozole (B) anastrozole (C) tamoxifen (D) exemestane (E) exemestane + everolimus (F) exemestane + trastuzumab	I	HR+, HER2- (A–E) or HER2+ (F) MBC No prior chemotherapy (A–E) or ≥1 chemotherapy (F)	65	AB (36 pts): DCR (duration NR): 67% C (16 pts): 75%		
Patnaik *et al*[22]	Abemaciclib Abemaciclib + fulvestrant	Phase I, 2 cohorts	≥ first line MBC 1 cohort unselected, median 7 prior therapies 2 cohort HR+ median 4 prior therapies	47 (36 HR+) 13	HR+: PR 36% (25% confirmed) PR 85% (62% confirmed)		
Dickler *et al*[24]	Abemaciclib	Phase II (MONARCH 1)	HR+, HER2- MBC Progressed on/after ET and chemotherapy (1–2 lines) Median 3 lines	132	RR (confirmed) 17.4% CBR (CR+PR+SD≥6 months) 42.4%	5.7	
Ribociclib							
Juric *et al*[25 26]	A1: Ribociclib + letrozole. A2: alpelisib + letrozole A3: Ribociclib + letrozole + alpelisib	Phase Ib/(II)	OR+,HER2-postmenopausal ABC ≥ first line in the dose expansion group	A1: 47 patients A2: 7 A3: 36 (27 evaluable)	A1: RR 5%, CBR (SD≥24 weeks) 32% (previously treated) 39%, CBR 73% (treatment-naïve) A2: Not relevant A3: PR 22% (15% confirmed), SD 22% non-CR non-PD 22%		
Bardia *et al*[27]	Ribociclib + everolimus + exemestane Ribociclib + exemestane	Ib/(II)	OR+, HER2- postmenopausal ABC	70 (55 evaluable) NR	CR 2%, PR 9% (4% confirmed) Disease control 71%*		

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.

Table 4 Grade 3 and grade 4 toxicities of CDK4/6 inhibitors

Reference	Therapy	Neutropenia (%)	Febrile neutropenia (%)	Leucopenia (%)	Anaemia (%)	Thromboctytopenia (%)	Fatigue (%)	Other (%)	Discontinuation (%)
Slamon *et al*[13]	Letrozole + palbociclib vs letrozole	17(grade 4) (≈ 2 DLT)	0	Common	-	-	Common	-	8 (dose interruption)
Clark *et al*[14]	Palbociclib + paclitaxel	59	4 (DLT)	-	-	-	-	Grade 3 AST/ALT(DLT)	67 (dose interruption)
DeMichele *et al*[49]	Palbociclib	51	3	51	5	22	0	Lymphopenia 30	3
Finn *et al*[16 36]	Letrozole + palbociclib Letrozole	51 1	0 0	19 0	6 1	2 0	4 1	-	13 2
Finn *et al*[17]	Letrozole + palbociclib Letrozole	All grades 79.5; grade 3: 56.1 All grades 6.3					37.4 27.5	Nausea 35.1 26.1	9.7 5.9
Turner *et al*[59], Cristofanilli *et al*[18]	Palbociclib + fulvestrant Placebo + fulvestrant ± goserelin	65. 1	1 1	28 2	3 2	2 0	2 1	-	4 2
Tolaney *et al*[23]	Abemaciclib + (A) letrozole (B) anastrozole (C) tamoxifen (D) exemestane (E) exemestane + everolimus (F) exemestane + trastuzumab	17					14	Diarrhoea 31 Nausea 6 Vomiting 3 Abdominal pain 3	
Patnaik *et al*[22]	Abemaciclib* Abemaciclib + fulvestrant	11 31		23			2 8	Diarrhoea 5 Nausea 3 Vomiting 2 Diarrhoea 8	
Dickler *et al*[24]	Abemaciclib						Common (number NR)	Common AE: diarrhoea, nausea, decreased appetite, abdominal pain number NR)	6.8%
Juric *et al*[25 26]	A1: ribociclib + letrozole. A2: alpelisib + letrozole A3: ribociclib + letrozole + BYL	43 (A1) 22 (A3)		2 (A1)	-	-	11 (A3)	Lymphopenia 4 (A1) Hyperglycaemia, 17 (A3) Nausea, 6 (A3)	NR (A1) 22(A3)
Bardia *et al*[27]	Ribociclib + everolimus + exemestane Ribociclib + exemestane	45.7		8.6		5.7		6 DLT: 1 febrile neutropenia, 2 ALT elevations, 2 thrombocytopenia, 1 mucositis	2.9

*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).[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.[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 ≥6 months) 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 *CCDN1* 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.[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.[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%), leucopenia (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.[13]

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=0.031), 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.[17]

The double blind phase III PALAMO-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.[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.[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 0.0.36 to 0.59, p<0.0001).[18] In addition, global quality of life was generally maintained with palbociclib but detoriated 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.[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.[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.[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.[21] 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.[21] The phase I study was expanded to evaluate the efficacy of abemaciclib plus fulvestrant in HR-positive MBC.[22] 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%).[22] No grade 4 events were reported.[22]

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.[23] The most common grade 3 toxicities were diarrhoea 31%, neutropenia 17%, fatigue 14% and nausea 6%. No grade 4 events were reported.[23]

Phase II

The phase II MONARCH-1 study (NCT02102490) evaluated abemaciclib monotherapy in 132 patients with HR-positive, HER2-negative MBCs who had previouly 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.[24] The most common AEs (grade) were diarrhoea, fatigue, decreased appetite and abdominal pain. Totally 6.8% of the patients discontinued treatment due to toxicity.[24]

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.[25 26] At the time of presentation, 47 patients were enrolled in A1.[26] 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 73% among 28 treatment-naïve patients.[26] 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.[25 26] Totally, 36 patients were enrolled in A3.[26] Among 27 evaluable patients 7% had PR and 15% an unconfirmed PR.[26] The most frequent grade 3–4 AEs were neutropenia (22%), hyperglycaemia (14%), fatigue (11%) and nausea (6%).[26]

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.[27] 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%.[27]

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 capecitabine, 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.[10]

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.[28–31] In contrast, using an orthotopic brain tumour model Parrish *et al*[29] 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.[32]

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
Palbociclib								
NCT01684215	Palbociclib	I/II	OR+, HER2-Japanese, phase I: solid tumours Phase II: postmenopausal, OR+, HER2-	58	First line	None	DLT 1-year PFS	January 2017, recruiting
NCT01976169	Palbociclib + trastuzumab-DM1 (T-DM1)	I	HER2+, ABC, prior trastuzumab Rb-proficient (Rb normal and low p16in4a)	17	No criteria	No criteria	DLT	August 2015, recruiting
NCT023844239	Palbociclib (100 mg) + fulvestrant or tamoxifen Palbociclib (125 mg) + fulvestrant or tamoxifen ± LHRH agonist	II	HR+, HER2-postmenopausal, MBC or LABC	70	Previous treatment with PI3Kinhibitor mTOR inhibitor, no limitations in lines	≤2 prior lines	PD (16 weeks)	August 2017 (not initiated March 2015)
Eudract database 2011-005637-38	Palbociclib Palbociclib + ETto which the patients had progressed	II, TREnd randomised	Postmenopausal	50	≥1 line ET; PD on ET	≤1	Clinical benefit	NR
NCT02448420	Palbociclib + trastuzumab ± (letrozole)	II, PATRICIA	OR+ and OR-	138	≥2 lines of HER2-directed therapy	No criteria	PFS, 6 months	December 2019
NCT02297438	Palbociclib + letrozole Placebo + letrozole	III, PALAMO-4	Asian, OR+, HER2-postmenopausal,≥12 months from adjuvant NSAI	330	first line	None	PFS	October 2017, recruiting
NCT02028507	Palbociclib + exemestane Capecitabine	III, PEARL	Postmenopausal, MBC, resistant NSAI	348	First or Second line	≤1	PFS	January 2018
Ribociclib								
NCT02333370	Ribociclib + letrozole	Ib/II	HR+, HER2-Postmenopausal ABC	112	First line	None	PFS (phase II)	February 2021, recruiting
NCT01857193	Ribociclib + everolimus + exemestane Ribociclib + exemestane Everolimus + exemestane	Ib/II	OR+, HER2-postmenopausal, LABC or MBC, adj NSAI	185	First line	≤1	Phase Ib: DLT Phase II: PFS	May 2016, recruiting
NCT01872260	Ribociclib + letrozole BYL719 (PI3K-α inhibitor) + letrozole Ribociclib + BYL719 + letrozole	Ib/II	ER+, HER2-Postmenopausal, LABC or MBC,	300	Ib dose escalation: ≥ first line Ib dose expansion: first line II: first line	Ib dose escalation: 1 Ib dose expansion: 0 II: 0.	Phase Ib: DLT Phase II: PFS	May 2017, recruiting
NCT02088684	Ribociclib + fulvestrant Ribociclib + BYL719 + fulvestrant Ribociclib + BKM120 (PI3K-pan-inhibitor)+ fulvestrant	Ib/II	HR+, HER2-postmenopausal, LABC or MBC,	216	Ib:≤2 II:≤1	Phase Ib: ≥first line Phase II: ≥first line	Phase Ib: DLT Phase II: PFS	February 2019, recruiting
NCT01958021	Ribociclib + letrozole Placebo + letrozole	III, MONALEESA-2	Postmenopausal, ABC	650	first line	None	PFS	August 2017, recruiting
NCT02422615	Ribociclib + fulvestrant Placebo + fulvestrant	III, MONALEESA-3	HR+, HER2-Postmenopausal ABC	660	first or second line	None	PFS	May 2020, not yet open
NCT02278120	Ribociclip + anastrozole/tamoxifen + goserelin Placebo + NSAI/tamoxifen + goserelin (double-blind)	III, MONALEESA-7	HR+, HER2-ABC, premenopausal or perimenopausal	660	first line	None	PFS	February 2018, recruiting
Abemaciclib								
NCT02107703	Abemaciclib + fulvestrant Placebo + fulvestrant	III, MONARCH 2	Postmenopausal, LABC or MBC, HR+, HER2-	630	First or Second line	None	PFS	February 2020, recruiting
NCT02246621	Abemaciclib + anastrozole/letrozole Placebo + anastrozole/letrozole	III, MONARCH 3	HR+, HER2-postmenopausal LABC or MBC, HR+, HER2-	450	First line	None	PFS	July 2021, recruiting
NCT02057133	Abemaciclib + letrozole Abemaciclib + anastrozole Abemaciclib + tamoxifen Abemaciclib + exemestane Abemaciclib (two doses) + exemestane + everolimus Abemaciclib (two doses) + trastuzumab LHRH agonist Doses NR	I	MBC, HR+, HER2- or HER2+ (trastuzumab)	102	First line ≥ second line depending on combination	≥1	Number with drug-related AE	November 2016, recruiting
Eudract number 2014-004010-28	Abemaciclib ± ET ± trastuzumab	II	HR+, brain metastases	120	No criteria	No criteria	Inracranial 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.[33 34] 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.[35]

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.[19 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.[37]

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.[39 40]

HER2-positive disease

A synergistic effect was seen in a preclinical study when treating HER2-positive cell lines with trastuzumab and palbociclib simultaneously.[4]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.[42] Additionally, in models of acquired resistance to HER2-targeting therapies palbociclib blocked proliferation and seemed to act synergistically with trastuzumab and T-DM1.[33 43 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 chemotherapeuticum. 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 46] 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.[47]

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).[14] 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.[25] 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.[50] Loss of Rb expression has been found to occur in 20–30% of BCs.[51] However, the incidence of Rb loss was dependent on the clinical subtype and was more common in triple-negative BC compared with other subtypes.[52] 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.[53] 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.[54 55] Especially, *CCND1* amplification was frequent in luminal tumours, albeit most notably in luminal B.[56]

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).[33 41 44 47] 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.[41] 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.[57] 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.[16]

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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