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CDK4/6 inhibitors in the treatment of patients with breast cancer: summary of a multidisciplinary round-table discussion

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ABSTRACT

This article is the result of a round-table discussion organised by ESMO Open in Vienna in December 2017. Its purpose is to discuss the background and advances in the evidence regarding cyclin-dependent kinase 4/6 inhibitors (palbociclib, ribociclib and abemaciclib) in the treatment of metastatic and early-stage breast cancer and to explore what the key open research questions are and next steps should be.

INTRODUCTION

Hormone receptor (HR)-positive breast cancers (BCs), which represent up to 75% of invasive BCs, are characterised by the presence of receptors for oestrogen (ER) and/or progesterone (PgR). Endocrine therapy (ET) remains the therapeutic backbone for the treatment of HR-positive patients with BC.[1] However, up to 50% of HR-positive patients with metastatic breast cancer (MBC) develop mechanisms of therapeutic resistance to the ET they receive.[2] In order to modify the development of resistance to ET thereby enabling patients to receive effective HR-directed treatments, inhibitors of the cyclin-dependent kinases (CDK) 4 and 6 have been developed and have recently shown a clinically meaningful efficacy and a good tolerability profile in patients with MBC.

Loss of cell cycle regulation leading to uncontrolled cellular proliferation is a hallmark of cancer.[3] In the cell cycle, the progression from G1 (pre-DNA synthesis) to S (DNA synthesis) is a critical checkpoint in preventing the cell from abnormal proliferation. A key regulator of this process is the cyclin D-CDK4/6-inhibitor of CDK4 (INK4)-retinoblastoma (Rb) pathway which has emerged as a promising therapeutic approach in cancer.[4] Initial clinical experience with first-generation pan-CDK inhibitors has shown poor efficacy and important toxicity.[5] However, a new generation of highly selective CDK4/6 inhibitors including palbociclib, ribociclib and abemaciclib has overcome the challenges of non-specific pan-CDK inhibitors and targets tumour types in which the cyclin D-CDK4/6-INK4-Rb pathway has a key role, more effectively.[6]

Basics and rationale of CDK4/6 inhibition in general and in BC in particular

The CDK4/6-cyclin D1 complex phosphorylates Rb1 leading to a loss of repression of E2F transcription factors, resulting in cell cycle progression from G1 to S phase and then in cancer proliferation. CDK4/6 inhibitors, while blocking CDK4/6-cyclin D1 complex, prevent phosphorylation of Rb1, stop the cell cycle progression from G1 to S phase (figure 1).[7]

Several cancers such as melanoma, glioblastoma, head and neck cancer, non-small-cell lung cancer and BC have alterations that activate the cyclin D-CDK4/6-INK4-Rb pathway.[8–11] In BC, alterations in the cyclin D-CDK4/6-INK4-Rb pathway are frequent. Amplification of the gene encoding cyclin D1 (*CCND1*) has been identified in about 15%–20% of human BC while overexpression of the protein has been demonstrated in a higher percentage.[12–15] Loss of the tumour suppressor P16 has been found in up to 50% of BC.[16]

Preclinical studies have provided the rationale for the clinical development of CDK4/6 inhibitors in specific molecular subgroups of BC.[17] [18] Cell lines representing the luminal ER-positive BC subtype (including human epidermal growth factor receptor 2 (HER2)-amplified cell lines with luminal features) were the most sensitive to growth inhibition by palbociclib and ribociclib.[18] [19]
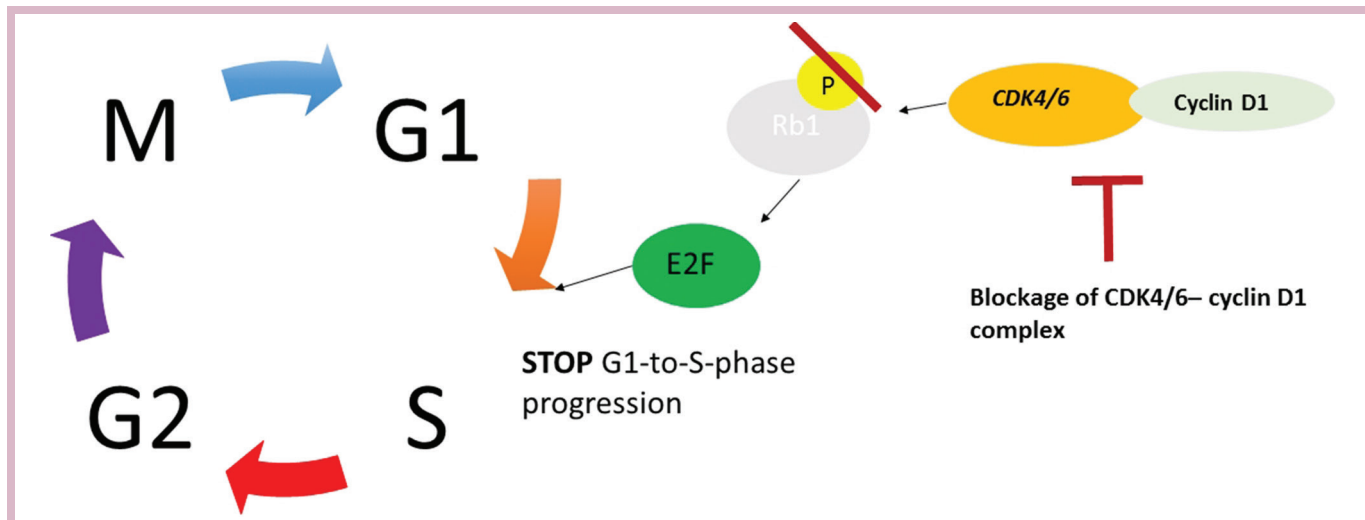
Figure 1 Cell cycle progression in cancer and the role of cyclin-dependent kinases (CDK)4/6 inhibitors. Adapted with permission of John Wiley & Sons from: Murphy[7]. Permission conveyed through Copyright Clearance Center.

By contrast, non-luminal or basal subtypes were shown to be the most resistant to palbociclib.[18 19]

In HR-positive BC, ER signalling upregulates cyclin D1 levels and potentiates multiple signalling pathways, resulting in upregulation of CDK4/6 activity.[4 20]

In addition, cyclin D1 facilitates ER transcriptional activity supporting the dependence of cyclin D1 to initiate cell cycle progression from G1 to S phase and to drive cancer cell proliferation, reinforcing the rationale for the combination of ET with CDK4/6 inhibitors.

Endocrine resistance in hormone-positive BC

Despite their name not all hormone responsive tumours are truly sensitive to ETs that inhibit the ER pathway. About 20% of HR-positive MBC are refractory to first-line ET (de novo or primary endocrine resistance).[21] Although clinicians usually treat HR-positive MBC patients with further ET after initial response to first-line therapy, the clinical benefit rate declines from 70% at first-line treatment to about 30% for second and further lines representing acquired or secondary endocrine resistance.[22]

A possible definition of primary and secondary resistance is provided in table 1[1] with the definition based on clinical experience and not on biology.

Acquired resistance to ET occurs due to the loss of ER functionality or loss of previously present ER dependence.

Loss of ER is infrequent and observed in only 10% of MBCs[23 24] and in <10% of BCs during neoadjuvant chemotherapy.[25] An increased genetic instability further complicates tumour heterogeneity, even though not all newly acquired mutations may be important for drug resistance. Besides tumour heterogeneity, dormancy with the risk of experiencing a late recurrence, and mutations in the gene *ESR1,* which encodes ERα, are further mechanisms of endocrine resistance. Moreover, overexpression and/or amplifications of growth factor receptors are associated with the appearance of endocrine resistance[23] and cell cycle checkpoint alterations can also contribute to loss of endocrine responsiveness.[7]

Mutations in *ESR1* are rare in untreated patients and were not identified by the Cancer Genome Atlas analysis as untreated primary BC specimen were analysed in this work.[26] Activating mutations of *ESR1*[27 28] are likely a mechanism of resistance to ET and were found in patients that had been treated with ETs.

However, in metastatic and pretreated BC, *ESR1* mutations occur more frequently. *ESR1* mutation status in cell-free tumour DNA (ctDNA) showed high concordance with contemporaneous tumour biopsies[29] and liquid biopsy has been used to detect *ESR1* mutations which could identify patients who have become resistant to particular ETs.[30] Clalot and coauthors found *ESR1* mutations in ctDNA in 75% of blood samples 3 and 6 months before progression on first-line therapy with aromatase inhibitors (AIs).[31] In recently published clinical data from two large randomised phase II trials, the PALOMA-3 and the SoFEA study, *ESR1* mutations were found in 29% and 39% in specimens from patients who received prior AI therapy, respectively. In comparison to treatment-naive patients with a very low rate of *ESR1* mutations, these data are in line with the existing evidence that *ESR1* mutations emerge more commonly with an acquired resistance after AI treatment.[32]

Table 1 Endocrine resistance in metastatic breast cancer (MBC)[1]

Primary endocrine resistance	Relapse within 2 years of adjuvant ET PD within first six months of first-line ET for MBC
Secondary endocrine resistance	Relapse while on adjuvant ET but after the first two years or relapse within 12 months after completing adjuvant ET PD≥6 months after initiating ET for MBC

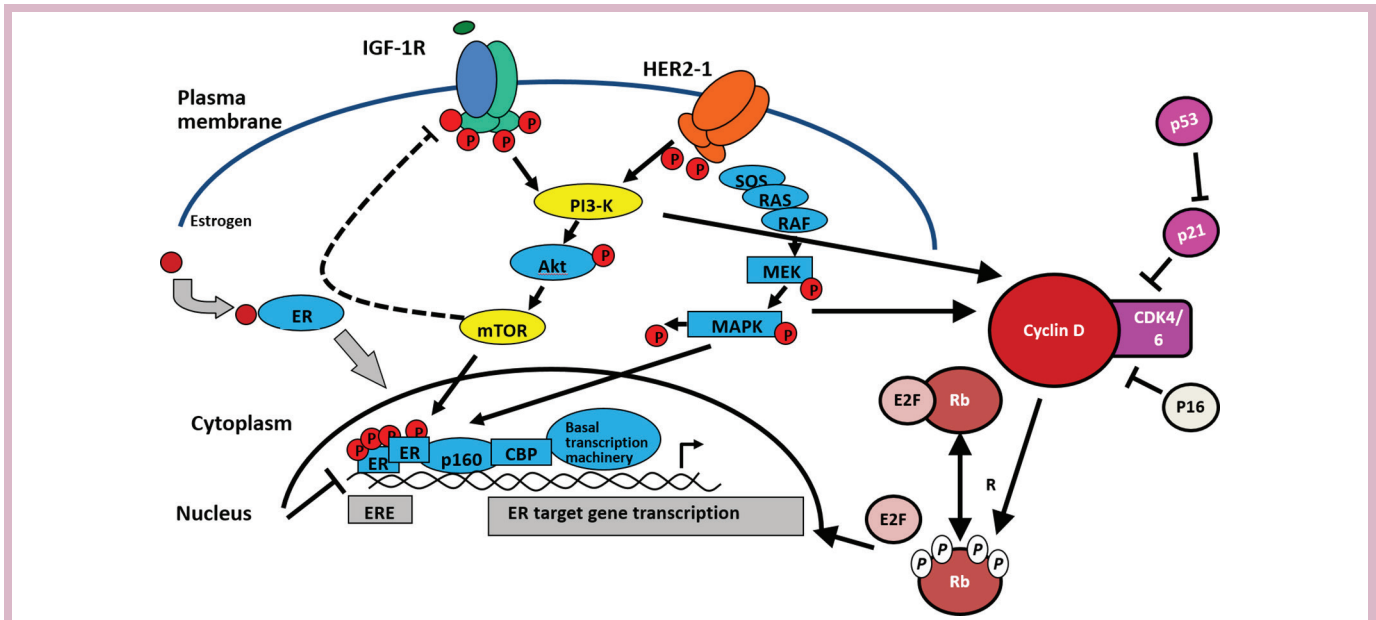ET, endocrine therapy; PD, progressive disease.

Figure 2 Cross-talk between oestrogen (ER) and epidermal growth factor receptor (EGFR)/insulin-like growth factor 1 receptor (IGF-1R) signalling pathway and cyclin-dependent kinase (CDK)4/6 function. Adapted with permission from Springer Nature: Di Cosimo S and Baselga J.[65] Copyright 2010. HER2-1, human epidermal growth factor receptor 2; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase.

Components of the growth factor receptor pathways including fibroblast growth factor receptor 1, HER2, HER3, epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor (IGF-1R) converge on the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) and Raf/mitogen-activated protein kinase/ERK kinase/extracellular-signal-regulated kinase (RAF/MEK/ERK) pathways and are frequently mutated in BC. These pathways regulate cell survival and proliferation and aberrations in the PI3K signalling pathways and lead to a pathway hyper-activation that promotes ER-independent ER transcriptional activation (figure 2).

The aberrations include both mutations of PIK3 catalytic alpha polypeptide (*PIK3CA*), *AKT1, AKT2* and *PDK1* and loss of inhibitory signals *PTEN* and *INPP4B* that occur in about 70% of BCs.[33] Blockage of PI3K pathway alteration results in a disturbed cross-talk and consecutively in an increased EER dependence that provides the rationale for an ET.[34]

The recently presented results of the phase II LORELEI trial (NCT02273973) showed a promising piece of evidence for the combination of PI3K inhibitor and non-steroidal aromatase inhibitor (NSAI) in the neoadjuvant setting as the combination of PI3K inhibitor taselisib with letrozole led to an improved objective response rate (ORR) (OR 1.55; 95% CI 1.00 to 2.38, p=0.049 for all and OR 2.03; 95% CI 1.06 to 3.88, p=0033 for PIK3CA mutations compared with letrozole alone).[35]

mTOR plays a key role in the regulation of protein translation, cell growth and metabolism. It exists in two distinct protein kinase complexes, that is, mammalian target of rapamycin complex (mTORC) 1 and 2.[36]

Phosphorylation of AKT leads to an increased mTORC1 kinase activity that promotes protein synthesis. It is another target that can be blocked to reverse emerging endocrine resistance. The concept of a mTOR blockade was successfully proven in the BOLERO-2 study, a phase III trial that compared the mTOR inhibitor everolimus in combination with exemestane versus exemestane with placebo in postmenopausal women with ER-positive, HER2-negative advanced breast cancer whose disease recurred during or within 12 months after the end of adjuvant treatment or progressed during or within 1 month after the end of treatment for advanced disease. The combination of everolimus and exemestane led to an improvement of progression-free survival (PFS) by 4.1 months (6.9 vs 2.8 months).[37]

In the recently presented results of the MANTA trial, the dual mTOR inhibitor vistusertib was inferior to everolimus.[38]

The cyclin D-CDK4/6-INK4-Rb pathway plays a key role in cell cycle regulation as it is downstream of multiple mitogenic cascades, making it a further important target for overcoming endocrine resistance.[39] Cyclin D associates with and activates the protein kinases CDK4 and CDK6 that have been associated with poor response and resistance to ET. Cyclin D1 amplification is a common event in ER-positive BC, identified in 58% of luminal B cancers and 29% of luminal A cancers.[26] Therefore, CDK4/6 inhibition is currently one of the most promising approaches to overcome endocrine resistance. In big trial programmes, CDK4/6 inhibition such as the PALOMA trials for palbociclib, the MONALEESA trials for ribociclib and the MONARCH trials for abemaciclib the efficacy of a CDK4/6 inhibition was evaluated. The

trials included patients with endocrine sensitive and in the PALOMA-3 and MONARCH2 trial also endocrine-resistant disease.

State of the art of CDK4/6 inhibitor efficacy in advanced HR-positive BC

Palbociclib

The first-in-class, oral CDK4/6 inhibitor palbociclib has been evaluated in several randomised clinical trials including patients with metastatic HR-positive, HER2-negative BC: the PALOMA-1/TRIO-18 trial was an open-label, phase II study that randomised 165 postmenopausal women with advanced HR-positive/HER2-negative BC in first-line therapy to receive either letrozole or letrozole plus palbociclib.[40] The study had two sequentially accrued cohorts: cohort 1, including patients with HR-positive/HER2-negative BC, and cohort 2, including patients bearing either cyclin D1 gene (CCND1) amplification or loss of p16, or both. The addition of palbociclib to letrozole significantly improved PFS compared with letrozole alone (20.2 vs 10.2 months, respectively HR, 0.488; p=0.0004).[40] The most frequently observed adverse event (AE) was grade 3–4 neutropenia (54% in the experimental arm vs 1% in the control arm).[40]

The PALOMA-2 trial confirmed the superiority of palbociclib and letrozole over letrozole for treatment-naive patients with HR-positive/HER2-negative MBC within the frame of a prospective randomised phase III clinical trial.[41] In total, 666 postmenopausal women were randomly assigned in a 2:1 ratio to receive letrozole with palbociclib or placebo, respectively. The median PFS was 24.8 months in the palbociclib arm versus 14.5 months in the placebo arm (HR 0.58; p<0.001).[42] Again, grade 3 or 4 neutropenia was the most common AE (66.4% in the palbociclib arm vs 1.4% in the placebo arm), with 1.8% rate of febrile neutropenia in the experimental arm and none in the control arm.[41]

The phase III PALOMA-3 trial randomised 521 women with pretreated HR-positive/HER2-negative MBC—regardless of menopausal status—to receive either fulvestrant plus palbociclib or fulvestrant plus placebo.[42] The combination of palbociclib and fulvestrant significantly improved PFS (9.2 vs 3.8 months, HR 0.42; p<0.001). The most common AE was again grade 3/4 AE neutropenia (62.0% in the palbociclib arm vs 0.6% in the placebo arm).[42] However, febrile neutropenia occurred in only 0.6% of patients who received palbociclib and in 0.6% of patients who received placebo. At the final analysis of the PALOMA-3 trial, the median PFS was 9.5 months in the palbociclib arm and 4.6 months in the placebo arm (HR 0.46, p<0.0001). The addition of palbociclib was superior in all subgroups.[43]

Based on these results, palbociclib has been approved by the US Food and Drug Administration (FDA) and European Medicines Agency for clinical use in the metastatic setting in combination with letrozole in patients with newly diagnosed BC or with fulvestrant in patients pretreated with ET.

Very recently, the first overall survival (OS) data from a randomised trial of palbociclib plus ET were reported. Namely, in the PALOMA-1 trial, median OS was 37.5 months for palbociclib/letrozole versus 34.5 months for letrozole (HR 0.897, p=0.281).[44] Median OS was 37.5 versus 33.3 months (HR 0.837, p=0.280) in cohort 1 and 35.1 versus 35.7 months (HR 0.935, p=0.388) in cohort 2, respectively. OS data from the larger PALOMA-2 trial are awaited.

Ribociclib

Ribociclib is another selective CDK4/6 inhibitor in the therapeutic armamentarium for BC.

The phase III MONALEESA-2 trial randomised 668 postmenopausal women with newly diagnosed HR-positive/HER2-negative MBC to receive either letrozole plus ribociclib or placebo. The experimental treatment resulted in a significantly improved PFS: at a median follow-up of 18 months, PFS was 63.0% in the ribociclib arm and 42.2% in the placebo arm.[45] The median duration of PFS was 14.7 months (95% CI 13.0 to 16.5) in the placebo group (HR 0.56; 95% CI 0.43 to 0.72, p=3.29×10^{-6} for superiority) versus not reached in the ribociclib group (95% CI 19.3 to not reached). Moreover, ribociclib led to a higher ORR compared with the letrozole/placebo (52.7% vs 37.1%, p<0.001). The most common AE was grade 3/4 neutropenia (59.3% in the ribociclib arm vs 0.9% in the placebo arm).[45]

The MONALEESA-7 is the first phase III trial dedicated to the evaluation of a CDK4/6 inhibitor as first-line therapy for premenopausal and perimenopausal patients with HR-positive/HER2-negative MBC.[44] In this study, the addition of ribociclib to either tamoxifen or a NSAI plus goserelin significantly improved PFS over placebo plus tamoxifen/NSAI plus goserelin (23.8 vs 13.0 months, respectively, HR 0.553, p=0.0000000983).[46] Treatment benefit was consistent across patient subgroups and regardless of endocrine agent.[46]

Abemaciclib

Abemaciclib is another potent selective CDK4/6 inhibitor with more pronounced inhibition of CDK4.[47] Equally to the other CDK4/6 inhibitors, the greatest benefit was observed among patients with HR-positive BC, with a disease control rate of 81% versus 33% for patients with HR-negative BC. Only patients with HR-positive BC experienced a treatment response. Heavily pretreated metastatic patients with HR-positive/HER2-positive and HR-positive/HER2-negative BC had a clinical benefit rate (CBR) of 54.5% and 64%, respectively.[48] In the HR-positive subgroup, median PFS was 8.8 months (95% CI 4.2 to 16.0).[48] Based on these results, abemaciclib has been granted FDA breakthrough therapy designation for patients with pretreated HR-positive MBC.

Abemaciclib monotherapy was assessed in the phase II single-arm MONARCH 1 study, which enrolled 132 women with HR-positive/HER2-negative MBC, whose disease progressed on or after ET and chemotherapy.[49]

Abemaciclib led to an ORR of 19.7%, CBR of 42.4% and median PFS of 6.0 months. The most common AEs all grades were diarrhoea (90.2%), fatigue (65.2%), nausea (64.4%), decreased appetite (45.5%) and abdominal pain (38.6%).

MONARCH 2 assessed abemaciclib plus fulvestrant in 669 women with HR-positive/HER2-negative MBC who had been pretreated with ET. These patients were randomised in a 2:1 ratio to receive either fulvestrant plus abemaciclib or fulvestrant plus placebo.[50] The combination of fulvestrant plus abemaciclib resulted in a significantly prolonged PFS over fulvestrant/placebo (16.4 vs 9.3 months, HR 0.553, p<0.001). Higher rates of ORR were achieved in the abemaciclib plus fulvestrant arm (48.1%) compared with the placebo arm (21.3%).[50] The double-blind phase III MONARCH 3 study randomised 493 postmenopausal women with HR-positive/HER2-negative advanced BC previously untreated in the advanced setting to receive either abemaciclib or placebo plus a NSAI. Median PFS was significantly longer in the abemaciclib arm (HR 0.54; 95% CI 0.41 to 0.72, p=0.000021; median: not reached in the abemaciclib arm, 14.7 months in the placebo arm). In patients with measurable disease, the ORR was 59% in the abemaciclib arm versus 44% in the placebo arm (p=0.004). In the abemaciclib arm, diarrhoea was the most frequent AE (81.3%) but was mainly grade 1 (44.6%). Comparing abemaciclib and placebo, the most frequent grade 3 or 4 AEs were neutropenia (21.1% vs 1.2%), diarrhoea (9.5% vs 1.2%) and leucopenia (7.6% vs 0.6%).[51]

Tables 2–4 give an overview on the phase III trials in patients with HR+/HER2 negative MBC.[41–43 45 51]

CDK4/6 inhibitors in early-stage BC
Several studies were recently initiated evaluating the potential role of CDK4/6 inhibitors in the neoadjuvant and adjuvant settings aiming at reducing the risk of BC recurrence:

Neoadjuvant setting
While preoperative ET is not commonly applied in patients with early stage BC, the combination of ET with CDK4/6 inhibitors offers the chance of applying neoadjuvant ET in a wider population, thereby offering the chance to forgo chemotherapy. In two studies, a major decrease in proliferation rate was demonstrated with the combination of palbociclib or ribociclib plus AIs.[52 53]

The French phase II NEOPAL trial directly compared standard neoadjuvant chemotherapy to ET plus palbociclib in 106 early-stage HER2-negative/luminal patients with BC. The percentage of patients with residual cancer burden (RCB) score 0/1 at surgery was defined as the primary study end point. While the rate of patients with RCB 0/1 was higher in the chemotherapy arm (15.5% vs 7.7%), response rates and breast conservation rates were comparable.[54] Finally, the phase II NeoMonarch trial evaluated abemaciclib as neoadjuvant treatment for early-stage HR-positive/HER2-negative BC. Overall, 223 postmenopausal women were included; patients received anastrozole, abemaciclib or the combination of both drugs for 2 weeks followed by abemaciclib plus anastrozole for an additional 14 weeks until surgery. The change in Ki-67 protein levels from baseline to week 2 was defined as primary study end point and this was significantly greater in patients receiving abemaciclib.[55]

Overall, these results suggest that further evaluation of CDK4/6 inhibitors as part of preoperative treatment concepts is warranted. Currently the NeoRHEA trial (NCT03065621), a single-arm phase II trial of neoadjuvant palbociclib plus ET, is recruiting premenopausal and postmenopausal patients. This study aims to identify novel biomarkers of palbociclib sensitivity or resistance.

Adjuvant setting
In addition to the neoadjuvant setting, two prospective randomised phase III trials evaluating the role of the

Table 2 Phase III trials with cyclin-dependent kinase4/6 inhibitors in hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer: median progression-free survival (PFS) and objective response rate

Trial	Line in the metastatic setting	Patients (n)	Treatment	HR for PFS	Median PFS, months	Objective response rate (ITT population) (%)
PALOMA-2	1L	666	Palbociclib + letrozole vs placebo + letrozole	0.58	24.8 vs 14.5	42.1 vs 34.7
PALOMA-3	>1L	521	Palbociclib + fulvestrant vs placebo + fulvestrant	0.46	9.5 vs 4.6	19 vs 8
MONALEESA-2	1L	668	Ribociclib + letrozole vs placebo + letrozole	0.56	NR vs 14.7	40.7 vs 27.5
MONARCH 3	1L	493	Abemaciclib + NSAI vs placebo + NSAI	0.54	NR vs 14.7	59 vs 44
MONARCH 2	1L or 2L	669	Abemaciclib + fulvestrant vs placebo + fulvestrant	0.55	16.4 vs 9.3	48.1 vs 21.3

ITT, intentiontion to treat; NR, not reached; NSAI, non-steroidal aromatase inhibitor.

Table 3 Phase III trials with cyclin-dependent kinase4/6 in hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer: safety data

Adverse event (AE)	PALOMA-2 Grade %			PALOMA-3 Grade %			MONALEESA-2 Grade %			MONARCH 3 Grade %			MONARCH 2 Grade %		
	All	3	4	All	3	4	All	3	4	All	3	4	All	3	4
Rash	18	1	–	NR	NR	NR	17.1	0.6	NR	NR	NR	NR	11.1	1.1	0
Fatigue	37	2	–	38	2	–	36.5	2.1	0.3	40.1	1.8	–	39.9	2.7	–
Diarrhea	26	1	–	19	–	–	35	1.2	–	81.3	9.5	–	86.4	13.4	0
Nausea	35	<1	–	29	–	–	51.4	2.4	–	38.5	0.9	–	45.1	2.7	–
Decreased appetite	15	1	–	12.8	0.9	–	18.6	1.5	–	24.5	1.2	–	26.5	1.1	0
Neutropenia	80	56	10	78.8	53.3	8.7	74.3	49.7	9.6	41.3	19.6	1.5	46.0	23.6	2.9
Anaemia	24	5	<1	26.1	2.6	0	18.6	0.9	0.3	28.4	5.8	–	29.0	7.0	0.2
Thrombocytopenia	16	1	<1	19.4	1.7	0.6	9	0.6	–	NR	NR	NR	15.6	2.0	1.4
Alopecia	33	–	–	14.8	–	–	33.2	–	–	26.6	–	–	15.6	–	–
QTcF Prolongation	–	–	–	–	-	-	3.3			–			–		
Increased creatinine	–	–	–	–	-	-	–	–	–	19	2.1	–	11.8	0.9	0

three different CDK4/6 inhibitors as adjuvant therapy when added to standard ET are currently ongoing (palbociclib: PALLAS, NCT02513394; abemaciclib: MonarchE, NCT03155997). In these trials focusing on intermediate and high-risk luminal patients, CDK4/6-inhibtor therapy is administered for a total duration of 2 years; of note, premenopausal patients may also be included when treated with additional gonadotropin-releasing hormone analogues.

Patients with poor response to neoadjuvant chemotherapy are at increased recurrence risk; therefore, the phase III PENELOPE-B trial (NCT01864746) investigates the addition of palbociclib for 1 year to standard ET as post-neoadjuvant therapy in patients with significant residual invasive disease after neoadjuvant chemotherapy. This study has recently finished accrual and results are eagerly awaited.

CDK 4/6 inhibition and rationale for their combination with other compounds beyond ET

CDK4/6 inhibitors with standard ET have changed the treatment of BC patients with metastatic HR-positive/HER2-negative disease. The antitumour activity with a favourable toxicity profile has been demonstrated in several phase III trials and is now the standard of care.[41 42 45 51] These results also led to the investigation of this class of with different combinations including:

Combination with anti-HER2 agents

CDK4/6 inhibitors overcome anti-HER2 resistance by increasing tumour cell dependence on EGFR family kinase signalling; therefore, CDK4/6 inhibitors may resensitise tumour cells to HER2-targeted therapies. Concomitant HER2 and CDK4/6 inhibition synergistically inhibits cell proliferation, controls tumour growth in vivo and delays tumour recurrence in transgenic mouse models.[56] There are several ongoing trials testing CDK4/6 inhibitors with different anti-HER2 drugs including trastuzumab, pertuzumab, neratinib, tucatinib and TDM1 (eg, NCT02530424; NCT02947685; NCT02448420; NCT02675231; NCT02657343; NCT03054363; NCT03054363).

Combination with PI3K/AKT/mTOR pathway inhibitors

Relapse of ER-positive BC is often associated with constitutive PI3K pathway activation. Targeting PI3K/mTOR and CDK4/6 pathways could be effective in ER-positive BCs and could overcome resistance to ER-targeted therapies. A combinatorial drug screen on multiple PIK3CA mutant cancers with decreased sensitivity to PI3K inhibitors revealed that combined CDK4/6-PI3K inhibition synergistically reduces cell viability. Importantly, the combination of PI3K and CDK4/6 inhibitors overcomes intrinsic and adaptive resistance to PI3K inhibitors leading to tumour regression in PIK3CA mutant xenografts.[57] There are several ongoing trials testing CDK4/6 inhibitors with different PI3K/mTOR inhibitors including copanlisib, taselisib, everolimus, vistursertib, pictilisib, gedatolisib and GDC-0077 (NCT03128619, NCT03006172,

Table 4 Phase III trials with cyclin-dependent kinase4/6 in hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer: inclusion criteria

	PALOMA-2 (%)		PALOMA-3 (%)		MONALEESA-2 (%)		MONARCH 3 (%)		MONARCH 2 (%)	
	Palbociclib + Letrozol	Placebo + Letrozol	Palbociclib + Fulvestrant	Placebo + Fulvestrant	Ribociclib + Letrozol	Placebo + Letrozol	Abemaciclib + NSAI	Placebo + NSAI	Abemaciclib + Fulvestrant	Placebo + Fulvestrant
Menopausal status										
Pre/perimenopausal	0	0	21	21	0	0	0	0	16	19
Postmenopausal	100	100	79	79	100	100	100	100	83	81
Gender										
Female	100	100	100	100	100	100	100	100	100	100
Male	0	0	0	0	0	0	0	0	0	0
Disease-free interval										
Newly metastatic	38	36	0	0	34	34	41	37	na	na
<12 months	22	22	5	2	1	3	na	na	na	na
>12 months	40	42	95	98	65	63	na	na	na	na
Treatment-free interval										
<36 months	na	na	na	na	na	na	28	40	na	na
≥36 months							63	50		
unknown							9	10		
Prior neo-/adjuvant chemotherapy	48	49	40	43	44	43	38	40	60	60
Prior therapies for advanced BC										
1	0	0	38	40	0	0	0	0	38	38
>2 (%)	0	0	38	34	0	0	0	0	0	0

na, not available; NSAI, non-steroidal aromatase inhibitor.

NCT02684032, NCT02389842, NCT02732119, NCT02871791, NCT02599714).

Combination with immunotherapy

In preclinical models, CDK4/6 inhibitors promote antitumour immunity by (a) increasing tumour antigen presentation plus suppressing the proliferation of regulatory T cells, and (b) promoting cytotoxic T-cell-mediated clearance of tumour cells. First, CDK4/6 inhibitors activate tumour cell expression of endogenous retroviral elements, thus increasing intracellular levels of double-stranded RNA and stimulating production of type III interferon, resulting in tumour antigen presentation. Second, CDK4/6 inhibitors markedly suppress the proliferation of regulatory T cells.[58] There are several ongoing trials testing CDK4/6 inhibitors with different PI3K/mTOR inhibitors including pembrolizumab and avelumab (eg, NCT02778685; NCT02779751; NCT03147287).

Combination with androgen blockade

Luminal androgen receptor (AR) represents a subgroup of triple-negative BC that may benefit from CDK4/6 inhibitors. However, the sensitivity to these inhibitors is correlated with the AR expression, and the absence/or low levels of cyclin E1 in cell lines.[59] Ongoing trials combining bicalutamide and CDK4/6 inhibitors are being conducted (eg, NCT02605486, NCT03090165).

CDK4/6 inhibitors in patients with brain metastases

Given the high efficacy and favourable tolerability of CDK4/6 inhibitors in MBC, this class of drugs may also constitute a novel treatment option for patients with brain metastases of BC. Differences in brain penetration between CDK4/6 inhibitors have been documented. P-glycoprotein and BC resistance protein have been shown to restrict the brain penetration of palbociclib, while abemaciclib crosses the blood–brain barrier more efficiently.[60 61] Ongoing clinical trials are evaluating the activity of palbociclib (NCT02774681) and abemaciclib (NCT02308020) in patients with brain metastases of BC.

Open questions regarding the best use of CDK4/6 inhibitors

CDK4/6 inhibitors with standard ET showed impressive improvement in PFS in first-line and second-line settings compared with ET alone. However, most of the patients with HR-positive tumours receive many lines of treatment over several years over the course of their disease. So far, no OS benefit with the addition of CDK4/6 inhibitors could be shown. However, the median follow-up is still short and further analysis needs to be awaited. Therefore, the real value of this new class of drugs cannot conclusively be judged yet. Several open questions accompany the discussion of their best use.

A key challenge is the search of patient populations that benefit most from the CDK4/6ET combination. The selection could be made clinically and/or biologically.

Several potential biomarkers such as TP53 mutation status, complexity of tumour genomics and PI3K/AKT/mTOR pathway alterations have been investigated and did not show any predictive value for treatment with CDK4/6 inhibitors.[62]

In all trials (first and second line), the different clinical subgroups including proliferation rate, number of organs involved, HR expression, age and race showed the same relative benefit by the addition of CDK4/6 inhibitors.

All these factors were prognostic, but not predictive. In the MONARCH 2 and 3 trials, an exploratory analysis of combined data of the first-line and second-line studies was performed showing the largest absolute difference in the populations with tumours with higher grading, with liver metastases, with PgR-negative disease and with a shorter treatment-free interval.[63]

Although these are signals, sequential trials are needed for better patient selection with the primary end point after two lines of treatment, particularly regarding the missing data about cross over after progression in the mono-endocrine arms.

Additionally, it is unclear what to do after progression on CDK4/6-ET combination therapy. One option—without much data so far—might be to change the backbone of ET only, for example, from an AI to fulvestrant while continuing CDK4/6 inhibition beyond progression. Another option could be drug holidays according to some preclinical data.[64]

Other important research areas regarding the use of CDK4/6 inhibitors include

Should they best be implemented in first-line or second-line treatment? A Dutch group has launched a phase lll sequential trial with the primary end point after two lines of treatment to solve this question (NCT03425838).

Does each patient need a combination ET from the beginning?

How does the existing combination ET with everolimus in second-line compare with CDK4/6 inhibitors? Also, the value of everolimus after failure of a CDK4/6 inhibitor is unclear and needs to be investigated.

Another research field will be the comparison of the CDK4/6 inhibitor-ET combination with upfront chemotherapy regarding the speed of response in patients with relevant visceral ER-positive disease.

Also, are CDK4/6 inhibitors able to replace chemotherapy in patients without a life-threatening disease (visceral crisis)?

If the efficacy of CDK4/6 inhibitors can be demonstrated in patients with brain metastases, further studies should focus on investigating the optimal sequence and combination of these drugs with other treatments used for brain metastases, such as stereotactic radiotherapy.

CONCLUSION

The use of CDK4/6 inhibitors with ET has changed the treatment landscape of BC patients with metastatic HR-positive/HER2-negative disease. Relevant antitumour activity with a favourable toxicity profile has been

demonstrated in several phase III trials. Consistently, all three drugs (palbociclib, ribociclib, abemaciclib) have shown a significant and clinically meaningful prolongation of PFS over ET alone, thereby establishing a novel treatment standard not only in pretreated patients but also in the first-line setting of HR-positive MBC. Initial results of studies evaluating the potential role of CDK4/6 inhibitors in the neoadjuvant setting suggest that further research is warranted in these patients.

With regards to CDK4/6 inhibitors in other combinations than with ET (ie, anti-HER2 agents, PIK3/AKT/mTOR inhibitors, immunotherapy and androgen blockers), trials are ongoing and results are awaited.

There are still many open questions in trial design, selection of patients and time points for the best use of CDK4/6 inhibitors that need to be addressed. One of the key challenges is the identification of predictive biomarkers for better patient selection. Sequential trials are needed.

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