

The role of abemaciclib in treatment of advanced breast cancer

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Abstract: Until recently, the mainstay of treatment in the majority of hormone receptor (HR)-positive, human epidermal growth factor 2 receptor (HER2)-negative advanced breast cancer (ABC) has consisted of single-agent endocrine therapy (ET). However, as understanding of endocrine resistance has grown, newer targeted agents have come to the fore. Inhibition of cyclin-dependent kinase complexes 4 and 6 (CDK4/6) combined with ET has shown significant activity in HR+ HER2- ABC, with impressive results in terms of progression-free survival (PFS) when compared with ET alone. This review summarizes the seminal findings pertaining to CDK4/6 inhibition in this population, specifically focusing on abemaciclib, contrasted with palbociclib and ribociclib. Potential directions for future studies are discussed, as a way of addressing outstanding issues such as establishing optimal treatment sequencing and agent combinations, appropriate patient selection to derive maximal benefits, predictive biomarkers and the employment of CDK4/6 inhibition beyond the ABC setting.

Keywords: abemaciclib, breast cancer, CDK4/6, ER positive, HER2 negative, metastatic, palbociclib, ribociclib

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Introduction

It is widely acknowledged that breast cancer (BC) is a molecularly diverse disease which can be divided into multiple different subgroups. In lieu of formal molecular definitions, BC is clinically divided according to tumour expression of receptors: hormone-receptor-positive (HR+) BC is characterized by positive expression of the oestrogen receptor (ER), progesterone receptor (PgR) or both; overexpression or amplification of human epidermal growth factor 2 receptor (HER2) bestows HER2-positive (HER2+) status; and an absence of HER2, ER and PgR defines triple-negative BC.¹ The vast majority of BCs are HR+, which in turn has established ET as an important foundation of both adjuvant and metastatic management of the disease. In HR+ HER2-negative (HER2-) metastatic or advanced BC (ABC), a common approach to treatment has, until recently, involved single-agent ET, with the exception of clinical scenarios involving high-risk features such as visceral crisis, or a

high burden of symptomatic disease, wherein induction treatment with cytotoxic chemotherapy to obtain rapid disease control is often the mainstay of initial management. However, discoveries into critical driver mutations in HR+ ABC, and the subsequent advent of targeted therapies, has recently changed this paradigm, wherein ET is now being paired with biological agents. Aberrations in phosphatidylinositol-3 kinase (PI3K), which lead to dysregulation of the mechanistic target of rapamycin (mTOR) signalling pathway, as is commonly seen in ABC, have been successfully targeted with the rapamycin analogue, everolimus. In the BOLERO-2 trial, in patients with HR+, HER2- ABC refractory to nonsteroidal aromatase inhibitors (NSAIs), the combination of everolimus with exemestane, a steroidal aromatase inhibitor, resulted in a median progression-free survival (PFS) of over double that of exemestane plus placebo,² but no statistically significant gain in overall survival (OS).³ Still

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maturing phase II data emerging from the BOLERO-4 trial show everolimus paired with letrozole to be an effective regime when given in the first-line setting to patients with HR+ HER2- ABC, though the median PFS is yet to be reached.⁴ Clinical trials into direct upstream targeting of PI3K with pan-PI3K inhibitors such as buparlisib, have thus far proven disappointing, with any observed benefit limited by significant toxicities.^{5,6}

More recently, inhibitors of cyclin dependent kinase complexes 4 and 6 (CDK4/6) have gained traction in the setting of HR+ HER2- ABC, with several seminal papers emerging showing compelling evidence for their use.

CDK 4/6 in the cell cycle

The mammalian cell cycle denotes a process of cell replication and is divided into four sequential phases. G1, the first growth phase, is succeeded by the S phase, in which deoxyribonucleic acid (DNA) synthesis takes place. Subsequent to that comes the second growth phase (G2), followed by the M phase of mitosis. Cell cycling is regulated by CDK complexes. Progression from G1 to S is dependent on CDK4/6, which work with D-type cyclins to hyperphosphorylate and inactivate the retinoblastoma tumour suppressor protein (Rb), which in turn releases the transcriptional factor E2F, upregulating the E2F-responsive gene, ultimately resulting in cell growth promotion. In ER+ BC, Rb1 gene function is usually preserved, and signalling dysregulation (by way of several potential mechanisms) of CDK4/6 activity is common, thus leading to cell cycling and proliferation.⁷ The therapeutic effect of ET relies in part on reducing the activity of the CDK4/6 cyclin D complex.⁸ Clinical resistance to ET often develops secondary to reactivation of the CDK4/6 pathway, which can occur through a number of different mechanisms (e.g. ligand-independent signalling and bidirectional cross talk between the ER pathway and growth factor signalling pathways and acquisition of oestrogen receptor 1 mutations).⁹⁻¹¹ As such, directly pursuing CDK4/6 represents a logical target for novel agents to be used in HR+ BC.¹² Selective inhibition of CDK4/6 by small-molecule tyrosine kinase agents causes the dephosphorylation of Rb, which in turn arrests cell-cycle progression in mid G1 phase, thus preventing ongoing proliferation of cancer cells.¹³

Therapeutic CDK4/6 inhibition

The first generation of small-molecule CDK inhibitors were less selective, with the second generation, being more specific to CDK4/6 inhibition, proving more potent.¹⁴ Currently, there are three CDK4/6 inhibitors that have reached clinical practice in the management of ER+ HER2- advanced disease; this review intends to provide an overview of all three, but with a specific focus on the data pertaining to abemaciclib.

Palbociclib

In 2014, phase II evidence in favour of palbociclib combined with letrozole *versus* letrozole alone in the first-line treatment of HR+ HER2- ABC was released, with the results of the PALOMA-1 study.¹⁵ The median PFS was nearly doubled in the combination arm; 20.2 months *versus* 10.2 months [hazard ratio 0.488, 95% confidence interval (CI) 0.319–0.748] for letrozole alone. This prompted the follow on of PALOMA-2, which tested the same cohort profile in a phase III setting. The median PFS was 24.8 months in favour of palbociclib plus letrozole *versus* 14.5 months for letrozole monotherapy.¹⁶ Finally, PALOMA-3 studied the effect of palbociclib plus fulvestrant in HR+ HER2- metastatic BC which had previously progressed on endocrine therapy (ET).¹⁷ Preclinical data had previously suggested that palbociclib could partially reverse acquired endocrine resistance in human BC cells *in vitro*,¹⁸ therefore the potential for therapeutic benefit to be derived from combined ET-CDK4/6 inhibition in the clinical setting was of particular interest. In this pretreated cohort, the median PFS was 9.5 months for the combination *versus* 4.6 months for fulvestrant alone. This benefit was shown to be consistent regardless of factors, including degree of previous endocrine resistance, HR expression level or phosphatidylinositol-3 kinase (PIK3CA) mutational status.

Ribociclib

Phase Ib preliminary data derived from an ER+ HER2- cohort showed promising response rates when ribociclib, a small molecule inhibitor of CDK4/6, was given in conjunction with letrozole.¹⁹ In patients naïve to treatment in the advanced setting, this combination had an objective response rate (ORR) of 46% and clinical benefit rate (CBR) of 79%. The development of ribociclib proceeded quickly to the phase III study of MONALEESA-2,

which trialled ribociclib with letrozole *versus* letrozole plus placebo in postmenopausal women with HR+ HER2- ABC who had not previously received systemic therapy for advanced disease.²⁰ Not unlike the results observed in PALOMA-2, PFS was significantly longer in the combination arm. At 18 months, the PFS rate was 63% (95% CI 54.6–70.3) in the ribociclib group, and 42.2% (95% CI 34.8–49.5) in the placebo arm. Median PFS was not reached in the ribociclib group and was 14.7 months in the placebo group. ORRs were in favour of ribociclib *versus* placebo (52.7% *versus* 37.1%, respectively). Updated efficacy data were reported after a median duration of 26 months, with confirmation of ongoing treatment benefit for the ribociclib group, and OS data are still to mature.²¹ MONALEESA-3, a phase III, double-blind trial, is yet to be reported. This study enrolled women with HR+ HER2- ABC who were either naïve to ET, or who had received only one line or prior ET; previous systemic cytotoxic chemotherapy in the advanced setting was not permitted.²² Randomization to the experimental arm of ribociclib plus fulvestrant *versus* fulvestrant plus placebo was stratified according to the presence of liver or lung metastases, and prior ET. The primary endpoint is PFS, secondary endpoints include overall survival, overall response rate and safety, as well as exploratory endpoints such as molecular alterations in tumour biopsy and circulating tumour DNA.

MONALEESA-7 is the first trial showing positive effect of CDK4/6 inhibition in a pre- and perimenopausal cohort with ABC. Ribociclib was used in tandem with either an NSAID plus goserelin or tamoxifen, *versus* ET alone. The study met its PFS endpoint, with a median PFS of 23.8 months in the ribociclib arm, compared with 13.0 months for ET alone. Additionally, the ORR was more favourable in the experimental arm compared with ET alone (51% *versus* 36%, respectively).²³

Abemaciclib

Preclinical development and evidence

Abemaciclib is the third small-molecule orally bioavailable inhibitor of CDK4 and CDK6 to be successfully developed from preclinical into clinical practice. It is structurally different to palbociclib and ribociclib, in that it exhibits a greater selectivity for CDK4 *in vitro*.²⁴ One group successfully demonstrated the ability of abemaciclib to exert CDK4/6 inhibition and subsequent cell arrest in mice bearing human colorectal xenografts, as well as demonstrating ensuing tumour

growth inhibition,²⁵ later validating those findings in human melanoma xenografts. This study supported the therapeutic dose and continuous dosing strategy employed in clinical studies of abemaciclib, demonstrating that steady-state trough plasma concentrations were achieved by continuous dosing, which maintained durable cell-cycle arrest. Rodent xenograft studies by another group showed abemaciclib can cross the blood–brain barrier, with active levels reached more efficiently, and at lower doses, than with palbociclib.²⁶

The *in vitro* and *in vivo* activity of abemaciclib in HR+ human BC models was recently characterized, with single-agent abemaciclib decreasing levels of phosphorylated Rb in BC cells.²⁷ Subsequent cell-cycle arrest in G1, secondary to decreased phosphorylated Rb levels, led to decreased observed proliferation overall. These corresponding decreases in cell cycle progression were shown to be sustainable and maintained beyond drug removal, with HR+ cell lines demonstrating a greater reduction in DNA synthesis upon prolonged exposure to abemaciclib compared with HR- lines. Another group has endeavoured to compare abemaciclib with palbociclib to define biological specificity in the preclinical setting.²⁸ Whilst cell-cycle inhibition was found to be dependent on Rb in both abemaciclib and palbociclib, abemaciclib was also observed to induce cell death in Rb-deficient cell lines.

Clinical trials

In vitro, cytochrome P4503A (CYP3A) accounts for over 99% of cytochrome P450-mediated metabolism of abemaciclib and its active metabolites. One group conducted several clinical studies into the interactions of abemaciclib with rifampin, a potent CYP3A inducer, and clarithromycin, a CYP3A inhibitor.²⁹ Abemaciclib was extensively metabolized (cleared predominantly by hepatic metabolism), with less than 10% of the parent drug detected in unchanged form in the faeces. The parent drug plus three active metabolites were detected in plasma and studied for interaction. Rifampin and abemaciclib coadministration (compared with abemaciclib alone) was shown to decrease abemaciclib AUC(0–?) and peak serum concentration (C_{max}) by 95% and 92%, respectively. Similarly, the AUC(0–?) and C_{max} of total active species (defined by the sum of abemaciclib plus the three active metabolites) decreased by 77% and 45%, respectively, in the setting of abemaciclib/rifampicin coadministration. Coadministration of clarithromycin

plus abemaciclib, compared with abemaciclib alone, increased abemaciclib AUC(0-∞) by 237%, and C_{max} by 30%. Total active species AUC(0-∞) increased by 119%, and C_{max} decreased by 7%. Correspondingly, the mean half-life of abemaciclib was prolonged from 28.8 to 63.6 h. In the light of this evidence, concomitant use of abemaciclib and CYP3A inducers and inhibitors, or other agents metabolized by CYP3A (e.g. sirolimus, cyclosporine, fentanyl) should be avoided or closely monitored.

A phase I, dose-escalation study of abemaciclib in patients with advanced cancer (including one cohort consisting of HR+ metastatic BC in combination with fulvestrant) successfully characterized the pharmacokinetic profile of the drug, with no identification of relevant covariates, suggesting dose adjustments according to patient weight, age or sex were not necessary.³⁰ Phase I data derived from heavily pretreated BC patients (with a median of seven lines of prior therapy), the majority of whom had visceral disease, showed activity of single-agent abemaciclib, with a response rate of 23% and median PFS of 5.8 months.³¹ This trial was not designed to compare response according to HR status, though note was made of a higher disease control rate in subjects with HR+ disease (81% versus 70% observed in the overall BC cohort, versus 33% in the HR- subgroup), with similar trends towards positive gains in response rate, PFS and median duration in response. This study also included a separate dose-escalation cohort which investigated once-daily and twice-daily dosing schedules using a 3 + 3 design. Dose-limiting toxicity (DLT) was not observed in subjects receiving once-daily abemaciclib at a prescribed maximum dose of 225 mg/daily, therefore the maximum tolerated dose (MTD) was not reached. In subjects enrolled to 12-hourly (q12 h) dosing, one of seven allocated to 200 mg q12 h experienced grade 3 fatigue DLT, with an increase to two of three subjects allocated to the 275 mg q12 h cohort reporting grade 3 fatigue. Thus, the recommended phase II trial dose was established at 200 mg q12 h. In subjects allocated to receive 200 mg q12 h, the median time from oral dose to maximum plasma concentration was 4 h (range: 0–10 h). Following multiple 200 mg q12 h doses, the mean area under the plasma concentration-time curve over 24 h at steady state reached 5520 ng·h/ml. The mean elimination half-life following a single oral administration of 200 mg was 21.3 h (range: 11.6–63.0). Single-agent abemaciclib was studied

in a Japanese population with advanced cancer, in order to evaluate its antitumour activity and pharmacokinetic profile in a non-White cohort. Investigators subsequently demonstrated an acceptable safety profile and observable antitumour activity at a dose of 200 mg twice daily.³¹

The first phase II study to report single-agent activity of abemaciclib was MONARCH-1, a single arm, open label trial which focused on patients with advanced HR+, HER2- BC with a history of previous progression on or following ET.³³ The population enrolled into MONARCH-1 represented a heavily pretreated cohort, with a median of three (ranging from one to eight) prior lines of systemic treatment, with a significant burden of metastatic disease. The majority (90.2%) had visceral metastases, with 70.5% represented by hepatic metastases, and just over a half had three or more metastatic sites of disease. Comparatively, only 2.3% had bone-only disease. Despite the setting of refractory disease in this study, the primary endpoint of ORR was 19.7%, with an observed clinical benefit rate of 42.4%. The median PFS was 6.0 months, and the median OS was 17.7 months.

MONARCH-2 was a phase III randomized, placebo-controlled trial which compared the efficacy and safety of abemaciclib plus fulvestrant versus fulvestrant plus placebo in women with HR+ HER2- ABC with evidence of endocrine resistance.³⁴ Selection criteria stipulated that eligible subjects have a history of progressive disease whilst receiving or within 12 months of completing neoadjuvant or adjuvant ET, or whilst being treated with ET in the first line for metastatic disease. One quarter of the entire studied population had primary endocrine resistance as defined by the investigators via the European Society for Medical Oncology guidelines,^{35,36} with the majority in each arm having received previous aromatase inhibitor therapy. Almost 60% had received chemotherapy in the adjuvant or neoadjuvant setting. Visceral disease was present in 55.7% of enrolled patients, and 26.9% had bone-only disease. The ORR for abemaciclib was 48.1%, versus 21.3% observed in the control arm. After a median 19.5 months of follow up, the median PFS in the abemaciclib arm was 16.4 months compared with 9.3 months (hazard ratio 0.553; 95% CI 0.449–0.681; $p < 0.001$).

The results of MONARCH-3 were published in 2017.³⁷ This double-blind, randomized phase III

study built on findings of its predecessors, this time focusing on postmenopausal women with HR+HER2- ABC who had not received systemic treatment in the advanced setting. Subjects who had received previous ET in the neoadjuvant or adjuvant setting were permitted, provided they had a durable response as evidenced by a disease-free interval of at least 1 year since therapy completion. Of those who had received prior ET, almost one half (47.8%) had experienced a treatment-free interval of 36 months or more. A total of 40% enrolled patients had received chemotherapy in the adjuvant or neoadjuvant setting. Abemaciclib plus an NSAI of physician's choice was compared with placebo plus NSAI in MONARCH-3, which represents a departure from MONARCH-2's comparator of fulvestrant, though the choice of NSAI lends comparative uniformity across trials, in that both PALOMA-2 and MONALEESA-2 mandated an NSAI as a combination partner and single-agent comparator with their respective CDK4/6 agents. Just over a half (53.3%) of MONARCH-3 patients had no history of previous NSAI use. *De novo* metastatic disease was represented in 39.7% of subjects. Visceral metastatic disease was reported in 52.9%, and 22.1% were recorded as bone only. Overall, the burden of metastatic disease in the enrolled population was considerable, with 46.4% reporting three or more disease sites. MONARCH-3 reported an ORR of 59% *versus* 44% in favour of the abemaciclib arm, with an associated significant prolongation in median PFS (hazard ratio 0.54; 95% CI 0.41–0.72; $p = 0.000021$, median not reached in abemaciclib arm *versus* 14.7 months in placebo arm). Data on OS are still immature and yet to be reported. A summary of the key characteristics of the published landmark PALOMA, MONALEESA and MONARCH trials can be found in Table 1.

A small study observed the objective intracranial response rate in women with HR+/HER- ABC with known brain metastases.³⁸ Abemaciclib produced partial responses in 2 out of 28 patients (8.7%), which has led to the opening of a second, larger phase II trial (see Table 2).

Issues of safety and toxicity observed with abemaciclib

Palbociclib, ribociclib and abemaciclib alike are available in oral form, and offer acceptable toxicity profiles. Palbociclib and ribociclib are administered daily, on a 3-weeks-on–1-week-off regime. Contrastingly, abemaciclib is dosed twice daily

on a continuous regime. Palbociclib and ribociclib report similar toxicity profiles, most notably neutropenia that can be ameliorated with dose reductions. Neutropenia occurred in 74–81% of subjects in the PALOMA trials, with 54–67% of those occurring at grade 3 and 4. Similar rates were observed in MONALEESA-2, with 74% overall incidence with 59% grades 3 and 4. The mechanism of action underlying neutropenia in the setting of CDK4/6 inhibition differs to that observed with cytotoxic chemotherapy; whilst the latter directly affects marrow cell precursors, CDK4/6 agents cause cell-cycle arrest with no apoptosis, thus, neutrophil counts recover quickly after the drug is withdrawn.^{39,40} An analysis of PALOMA-3 data suggested no diminishment in therapeutic efficacy as a result of protocol-mandated dose reductions in response to cytopaenias.⁴¹ Ribociclib has also infrequent incidences of prolongation of the QTc interval; a phenomenon that was not reported in either the PALOMA or MONARCH trials.

Contrastingly, in MONARCH-2, neutropenia occurred less often (46% overall; 27% occurring at grade 3 and 4), of which the majority were not associated with febrile neutropenia nor secondary infection. Notably, however, three deaths determined as related to the study drug did occur, with all three attributed to sepsis. The investigators implicated suboptimal guidance regarding the administration of granulocyte colony-stimulating factor (GCSF) and failure to follow directives regarding study drug dose reduction as contributing to two of those deaths. The remaining death was in the setting of immunosuppression secondary to exogenous steroid use for an intercurrent nonmalignant pathology. This underlines the importance of dose reduction to maintain safety in the setting of cytopaenias, as well as raising questions of practicality regarding the appropriate management of such in the clinical setting, wherein GCSF is often not approved or funded for palliative therapies.

Abemaciclib is classically characterized by a higher rate of fatigue and diarrhoea compared with palbociclib and ribociclib, possibly due to a greater selectivity for CDK4. First-in-human safety data of abemaciclib monotherapy reported diarrhoea (all grades) occurring in 63% of patients, with the majority (43%) at grade 1, 15% at grade 2, and 5% at grade 3, with no grade 4 events. Similarly, all-grade fatigue occurred in 41% of subjects (22%, 16% and 3% at grades 1,

Table 1. Key summary of published seminal clinical cyclin-dependent kinase complex 4/6 inhibitors.

	PALOMA-1¹⁵	PALOMA-2¹⁶	PALOMA-3¹⁷	MONALEESA-2²⁰	MONARCH-1³²	MONARCH-2³³	MONARCH-3³⁶
Trial phase	II	III	III	III	II	III	III
Treatment setting	First-line ABC	First-line ABC	Pretreated ABC	First-line ABC	Pretreated ABC	Pretreated ABC	First-line ABC
n (total population)	165	666	521	668	132	669	493
Intervention	P + L versus L	P + L versus L + placebo	P + F versus F + placebo	R + L versus L + placebo	Single arm, single-agent A	A + F versus F + placebo	A + NSAI versus NSAI + placebo
DFI on previous ET given in adjuvant or metastatic setting [% of total population]	>12 months, 33.3% <12 months, 17.6% <i>De novo</i> ABC, 49.1%	>12 months, 22.1% <12 months, 40.7% <i>De novo</i> ABC, 37.2%	<12 months, 100%	>12 months, 63.7% <12 months, 2.0% <i>De novo</i> ABC, 33.9%	NR	**Primary ER, 25.2% **Secondary ER, 73.1%	>36 months, 27.1% <36 months, 15.0% Unknown, 4.4% <i>De novo</i> ABC, 39.7%
Metastatic disease site [% of total population]	Visceral, 48.5% Nonvisceral (excluding bone only), 33.9% Bone only, 17.6%	Visceral, 48.6% Nonvisceral (excluding bone only), 28.7% Bone only, 22.7%	Visceral, 59.7% Nonvisceral (all), 40.3%	Visceral, 58.8% Bone only, 22.0%	Visceral, 90.2% Bone only, 2.3%	Visceral, 55.7% Bone only, 26.9% Other, 16.9%	Visceral, 52.9% Bone only, 22.1% Other, 25.0%
Median PFS of CDK4/6 + ET combination versus single-agent ET (months)	20.2 versus 10.2	24.8 versus 14.5	9.5 versus 4.6	Not reached versus 14.7	*6.0 (secondary endpoint)	16.4 versus 9.3	Not reached versus 14.7

*Single-agent study of trial drug; no control comparator arm.

**DFI not measured in MONARCH-2; endocrine resistance defined according to European Society of Medical Oncology consensus guidelines.

A, abemaciclib; ABC, advanced breast cancer; CDK4/6, cyclin-dependent kinase complexes 4/6; DFI, disease-free interval; ER, oestrogen receptor; ET, endocrine therapy; F, fulvestrant; L, letrozole; NR, not reported; NSAI, nonsteroidal aromatase inhibitor; P, palbociclib; PFS, progression-free survival; R, ribociclib.

Table 2. List of abemaciclib trials currently open to recruitment for subjects with breast cancer.

ClinicalTrials.gov identifier	Study phase	Population	Comparator	Primary outcome(s) measured
NCT03099174	I	HR+ HER2- ABC or mBC	Abemaciclib with xentuzumab (IGF mAb) +/- ET	MTD, DLT in the MTD, OR
NCT01655225	I (FIH)	HR+ HER2- ABC or mBC	Abemaciclib with LY3023414 (PI3K/mTOR dual inhibitor)	Recommended phase II dose
NCT02791334	Ia/Ib	HR+ HER2- mBC (pretreated)	Abemaciclib with LY3300054 (PD-L1 checkpoint antibody)	DLTs
NCT02784795	Ib	mBC with evidence of Notch pathway alterations	Abemaciclib with LY3039478 (Notch signalling inhibitor)	MTD
NCT02057133	Ib	HR+ HER2- mBC One arm for HR+ HER2+ mBC	Combination with other therapies (ET, everolimus, trastuzumab, LY3023414)	AEs
NCT02779751	Ib	HR+ HER2- mBC (pretreated)	Combination with pembrolizumab	AEs SAEs
NCT02831530	II	HR+ eBC prior to upfront surgery	Abemaciclib <i>versus</i> no treatment	Antiproliferative response
NCT02675231	II	HR+ HER2+ ABC or mBC following at least two lines of HER2 therapies for ABC	Abemaciclib plus trastuzumab +/- fulvestrant <i>versus</i> SOC plus trastuzumab	PFS
NCT02747004 (nextMONARCH-1)	II	HR+ HER2- mBC (pretreated)	Abemaciclib + TAM <i>versus</i> abemaciclib alone	PFS
NCT02675231 (monarchHER)	II	HR+ HER2+ locally advanced or mBC (pretreated)	Abemaciclib + trastuzumab +/- fulvestrant <i>versus</i> SOC chemotherapy (physician's choice)	PFS
NCT03130439	II	Rb positive, recurrent, locally advanced, unresectable or metastatic TNBC	Abemaciclib alone	ORR
NCT02308020	II	HR+ HER2+ or HER2- mBC with brain metastases	Abemaciclib (can receive intercurrent ET with or without trastuzumab)	OIRR (% of CR and PR)
NCT03155997 (MONARCHe)	III	HR+ HER2- node-positive eBC with high risk	Abemaciclib +ET <i>versus</i> ET alone	IDFS
NCT02763566 (MONARCHplus)	III	HR+ HER2- locally recurrent or mBC in postmenopausal women	Abemaciclib + fulvestrant <i>versus</i> fulvestrant + placebo and NSAI + abemaciclib <i>versus</i> NSAI + placebo	PFS

ABC, advanced breast cancer; AE, adverse event; CR, complete response; DLT, dose-limiting toxicities; eBC, early breast cancer; ET, endocrine therapy; FIH, first in human; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IDFS, invasive disease-free survival; IGF, insulin-like growth factor; mAb, monoclonal antibody; mBC, metastatic breast cancer; MTD, maximum tolerated dose; mTOR, mechanistic target of rapamycin; NSAI, nonsteroidal aromatase inhibitor; OIRR, objective intracranial response rate; OR, objective response; ORR, objective response rate; PDL1, programmed death ligand-1; PFS, progression-free survival; PI3K, phosphatidylinositol-3 kinase; PR, partial response; Rb, retinoblastoma protein; SAE, serious adverse event; SOC, standard of care; TAM, tamoxifen; TNBC, triple-negative breast cancer.

2 and 3, respectively), with no grade 4 events. These toxicities were reversible and occurred predominantly within the first 2 weeks of commencing treatment.³¹ The majority of patients in MONARCH-2 (86%) experienced diarrhoea, though only 13% were grade 3, with no grade 4 diarrhoeal events noted, and a small associated discontinuation rate (3%) due to this peculiar toxicity. Toxicities associated with combination abemaciclib and NSAI therapy in MONARCH-3 were in line with those observed in MONARCH-2, with the majority of adverse events occurring at grade 1 or 2 severity. Specifically, 81% of patients receiving abemaciclib plus ET in MONARCH-3 reported diarrhoea, 27% at grade 2, decreasing to 9.5% at grade 3, with no grade 4 events. MONARCH-3 incorporated a protocol-driven directive regarding the management of associated diarrhoea, instructing prompt commencement of antidiarrhoeal agents at the first onset, and subsequent dose reductions of abemaciclib for recurrent or high-grade episodes. Of the 27% of patients overall who initially reported grade 2 or 3 diarrhoea, 83.8% had no subsequent diarrhoeal events of the same or greater severity.

Arguably vital data yet to be reported in detail from all the seminal CDK4/6 trials pertain to quality-of-life indicators. In the setting of a population with incurable disease, wherein a gain in PFS, countered by a diminishment in functional status and perceived quality of life, may not be considered by patients to represent a worthwhile endeavour overall, contrasting the gain in PFS with patient-reported quality-of-life instruments is meaningful. This is perhaps most important when the most common toxicities observed in a drug have a greater potential to impact practical quality of life, such as is the case of abemaciclib and its unique propensity to trigger significant fatigue and gastrointestinal side effects. Comparatively, cytopenias, as are observed more commonly in ribociclib and palbociclib, are often asymptomatic and remedied by dose modifications, thus perhaps not impacting as significantly on quality of life. MONARCH-2 measured pain and symptom burden using the Brief Pain Inventory (BPI), and both MONARCH-2 and -3 gathered quality-of-life data *via* the EuroQol 5-dimension 5 level and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLC C30) and Breast 23 instruments, and their results are awaited with interest. Preliminary data pertaining to the effect diarrhoea and pain had on patient

fatigue as measured by the BPI and EORTC QLC C30 have been reported from the MONARCH-1 cohort.⁴² Diarrhoea was not a significant predictor of fatigue, but pain was found to be positively associated in patients undergoing third line or greater treatment.

Future directions for research and unanswered questions

Subgroup analyses: is there an 'ideal' group to receive upfront CDK4/6 inhibition?

As in PALOMA-2 and MONALEESA-2, cross over was not permitted in MONARCH-3, thus leaving unanswered the question as to whether upfront combination CDK4/6 plus ET in patients previously untreated in the advanced setting is superior to sequential single-agent ET followed by combination therapy. Exploratory subgroup analyses conducted within MONARCH-3 raise some indications that first-line combination therapy may not always be the ideal choice for all.³⁷ In patients with clinical indicators of good prognosis, namely: bone-only metastases, a preceding history of a prolonged treatment-free interval (>36 months), or an absence of liver metastases, endocrine monotherapy bestowed a comparatively better prognosis, perhaps suggesting that single-agent ET may represent an acceptable first-line option in such patients. Conversely, subjects with clinical markers of a poor prognosis, the presence of visceral with or without hepatic metastases alone, or a history of a short treatment-free interval (the latter of which, in the setting of MONARCH-3, may be regarded as a possible indicator of primary endocrine resistance), benefitted more from combination therapy. The MONARCH investigators have recently presented exploratory combined analyses of patients enrolled in both MONARCH-2 and MONARCH-3 that further support these hypotheses.⁴³ The greatest benefit, in terms of PFS and ORR, derived from combination abemaciclib plus ET was observed in patients with characteristics associated with a poor prognosis; namely, liver metastases, high-grade tumours or tumours that were PgR negative. Furthermore, a subpopulation treatment-effect pattern-plot analysis of MONARCH-3 subjects also confirmed that patients with a short treatment-free interval (<36 months) had a poorer prognosis and conversely, derived a significant improvement with abemaciclib and ET in the first line, compared with those with a treatment-free interval in excess of 36 months. Whilst PALOMA-2 and MONALEESA-2

reported a benefit favouring combination CDK4/6 inhibition with ET in all subgroups, there are limited data commenting on the absolute benefit in terms of PFS seen in the different subgroups with which to compare the data emerging from MONARCH-3. In PALOMA-1, the absolute benefit observed in the first 6–9 months of treatment was minimal in patients with bone-only disease, though the population of bone-only disease studied in this phase II context was very small, so should be interpreted with caution.⁴⁴ PALOMA-2 investigators have recently reported a hazard ratio of 0.36 (95% CI 0.22–0.59, $p < 0.001$) in the bone-only subgroup, with the median PFS not reached in the combination arm, *versus* 11.2 months for ET/placebo.⁴⁵ Again, Kaplan–Meier analysis showed the absolute benefit in the first ~9 months of combination treatment was minimal, perhaps suggesting upfront single-agent ET in this group would be a reasonable choice. To date, no subgroup analyses in terms of PFS have been reported from the MONALEESA trials.

Given the existence of strong, documented evidence of the activity of CDK4/6 inhibition in the second line and beyond, a lack of demonstrated overall survival (OS) benefit thus far in all seminal trials, and recent exploratory analyses that suggest more benefit in CDK4/6 in the front line in some groups more than others, there is currently no strong indication to recommend upfront combination CDK4/6 plus ET in all patients. Those with indolent metastatic disease may benefit from commencing ET alone, with the addition of CDK4/6 agents at a later point. Furthermore, whilst OS data represent a clinically relevant endpoint in the first-line management of ER+ HER2– ABC, they are arguably not the most sensitive or accurate measure of benefit derived from upfront treatment, given that postprogression survival may be largely reflective of lines of therapy subsequently received off study. Clearly, these issues represent areas that require more data from prospective trials in order to guide treatment strategies for patients with more favourable baseline characteristics.

Upfront CDK4/6 inhibition versus cytotoxic chemotherapy

Critically, it must also be noted that upfront combination CDK4/6 plus ET therapy has not yet been trialled against chemotherapy in the metastatic setting. In clinical practice, in recommending first-line systemic therapy for HR+ HER2– disease in the

context of heavy visceral disease burden or significant symptoms, cytotoxic chemotherapy is a common first choice of upfront therapy and as such, a comparison between this strategy *versus* CDK4/6 + ET regimes would address questions of sequencing. Similarly, in the event of CDK4/6 inhibition showing activity in the curative setting, the issue of the most appropriate choice and timing of treatment will inevitably arise again, should head-to-head studies not provide appropriate data.

Predictive biomarkers

Whilst perhaps intuitively logical, selecting patients for combination ET and CDK4/6 inhibition based on clinicopathological risk factors alone is likely to be improved significantly by way of the addition of predictive biomarkers. Both PALOMA-1 and 3 failed to show any association between palbociclib activity and cyclin expression, PIK3CA mutations or activating mutations in the oestrogen receptor 1 (ESR-1).^{11,16,17,46} Similarly, exploratory analyses of various biomarkers (including Rb characterization, p16, levels of Ki-67, cyclin D1, and ESR-1) collected in the MONALEESA-2 study failed to show any predictive power of response to ribociclib and letrozole.⁴⁷ Preclinical data suggest that effective CDK4/6 inhibition relies upon the presence of an intact and functional Rb protein,^{48,49} a phenomenon which is mostly commonly observed in luminal BCs.^{50,51} An Rb1 loss-of-function gene signature has been shown as a potential signature of sensitivity to CDK4/6 inhibitors.⁵² There are also encouraging early signs that thymidine kinase 1 (TK1), a cell-cycle-regulated enzyme that peaks in the S phase during DNA synthesis, may also serve as a potential biomarker, with one group demonstrating its utility in illustrating the pharmacodynamic effect of palbociclib in ER+ BC.⁵³ Another group has also reported TK1 levels as a response marker to palbociclib.⁵⁴

CDK4/6 inhibition: implications for resistance

As with other antineoplastic agents, emergent resistance to CDK4/6 inhibition over time will represent a therapeutic challenge to clinicians. The potential mechanisms of resistance in this context are still not well understood, though one group has recently hypothesized that uncoupling of the G1–S checkpoint in the cell cycle from growth factor or endocrine-mediated regulation may provide an important route of resistance to CDK4/6 inhibitors.⁵⁵ This group demonstrated that prolonged exposure of

two different hormone-sensitive cell lines models to abemaciclib led to the emergence of multiple independent clones with acquired CDK6 amplification and associated increase in CDK6 expression. This resulted in reduced response of phospho-Rb (pRb) to abemaciclib; with the resistant, CDK6 overexpressing cells requiring higher doses of abemaciclib to provoke cell-growth inhibition. Conversely, knockdown of CDK6 restored abemaciclib sensitivity. Inactivity of abemaciclib was observed in models with concurrent CDK6 overexpression and a mutation in Rb, and another with cyclin E1 overexpression. A correlation was also found between CDK6 amplification and a reduction in endocrine receptor messenger ribonucleic acid and protein expression, with an associated reduced responsiveness to ER antagonism. This prompts the question as to whether sequential ET following progression on a CDK4/6 inhibitor may not be the optimal approach in some patients, given a possible expectation of ongoing endocrine resistance in the setting of CDK6 overexpression.

Adaption to CDK4/6 inhibition *via* an alternate PI3K-dependent, D1-CDK2-mediated S-phase entry has also been described.⁵⁶ This study demonstrated the utility of combining CDK4/6 inhibition with other targeted agents that block expression of cyclin D1, and other G1-S cyclins. The combination of CDK4/6 inhibition with PI3K-targeted therapy *in vitro* and in patient-derived xenograft models resulted in tumour regression. Similarly, triple therapy combining ET, PI3K and CDK4/6 inhibition proved more efficacious again than doublet therapy. Although combined upfront PI3K and CDK4/6 inhibition was shown to prevent development of resistance, a PI3K inhibitor was not capable of restoring sensitivity, due to a loss of dependence on cyclin D1-CDK4/6 in acquired resistant cells, thus, supporting the potential for use of the combination of CDK4/6 and PI3K inhibitors in treatment-naïve tumours (wherein pRb proficiency and low levels of cyclin E1 expression would be anticipated). Similarly, a first-in-human, dose-finding phase I trial combining abemaciclib with a PI3K/mTOR dual inhibitor is currently open for enrolment (see Table 2).

CDK4/6 inhibition in the curative setting

As evidence in favour of CDK4/6 inhibition emerges in the metastatic setting, its utility in the neoadjuvant and adjuvant management of BC is being investigated. A pilot phase II adjuvant feasibility study of palbociclib given with continuous

ET in patients with stage 2 or 3 early HR+/HER2- BC (the majority of whom had completed prior adjuvant chemotherapy; all had completed 3–24 months of prior ET) has recently been reported.⁵⁷ The planned duration of treatment for this trial was 2 years, with the cumulative rate of all discontinuations of treatment being 15.1% at 6 months, and 20.9% and 27.8% at 12 and 18 months, respectively. Just over half of participating patients required dose reduction, with approximately one third permanently discontinuing palbociclib due to adverse events. This raises questions regarding the effect of dose reduction or interruption on CDK4/6 inhibition activity. Previous data arising from PALOMA-2 suggest that PFS was not negatively impacted by withholding of dose or dose reduction,⁵⁸ perhaps raising the possibility that lower doses of CDK4/6 inhibitors or a shorter duration of treatment may prove feasible that may in turn benefit overall rates of treatment adherence, a concern which is especially critical in the curative setting. Supporting this premise, preliminary pharmacodynamic data seem to suggest lower than recommended doses of CDK4/6 inhibitors may still be therapeutically active.^{59,60}

Interim results of neoMONARCH, a phase II study of abemaciclib as a neoadjuvant treatment in postmenopausal women with HR+ HER2- BC demonstrated reduction in tissue Ki67 levels was greater when abemaciclib was used as monotherapy or in combination with anastrozole, compared with anastrozole alone.⁶¹ MONARCHe, a trial examining the role of abemaciclib in high-risk early BC, is currently open to recruitment. Table 2 summarizes trials currently open and specifically recruiting patients with BC in the context of abemaciclib.

Conclusion

It is unlikely that data will ever be produced that directly compares abemaciclib with its contemporary agents palbociclib and ribociclib in order to ascertain superiority of one over the other. Nevertheless, this is not likely to be of much consequence, given that all three thus far demonstrate similar benefits in terms of PFS, with no OS benefit reported by any group as yet; and overall globally manageable safety profiles, ultimately the choice of drug is not likely to be as critical as the clinical context in which it is given. Palbociclib and abemaciclib both have proven ability in the first or second line of treatment of

advanced disease but given the lack of crossover in all of the landmark trials, as yet, it is not known whether sequencing may be critical to deriving the greatest benefit from CDK4/6 inhibition. Similarly, though subgroup analyses clearly show a global benefit from CDK4/6-targeted therapy, further analysis into the absolute (rather than proportional) PFS benefit derived in each group is warranted, in order to identify those in whom a delayed introduction to CDK4/6 inhibition may be the best approach. Predictive biomarkers are yet to be discovered that may guide clinicians as to the best timing of treatment initiation, as well as selecting patients who are most likely to benefit from CDK4/6-targeted therapy (and conversely, those who may safely proceed on ET monotherapy, with addition of a CDK4/6 agent at a later time point). As is inescapably the case in metastatic disease, eventual resistance to therapy is inevitable, and therefore future trials must not only focus on the issues of biomarkers, temporal sequencing and appropriate patient selection, but also be designed to address the likelihood of cross resistance. The place of CDK4/6 inhibition in the metastatic ER+ HER2- BC setting seems assured, yet there is still much to learn.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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