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BREAST CANCER (B OVERMOYER, SECTION EDITOR)

CDK 4/6 Inhibitors in Breast Cancer: Current Controversies and Future Directions

Laura M. Spring[1] · Seth A. Wander[1] · Mark Zangardi[1] · Aditya Bardia[1]

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Abstract

Purpose of Review To describe the clinical role of CDK 4/6 inhibitors in hormone receptor-positive (HR+) metastatic breast cancer (HR+ MBC) as well as current controversies and evolving areas of research.

Recent Findings Palbociclib, ribociclib, and abemaciclib are each approved in combination with an aromatase inhibitor or fulvestrant for HR+ MBC. Abemaciclib is also approved as monotherapy for pre-treated patients. Key questions in the field include whether all patients with HR+ MBC should receive a CDK 4/6 inhibitor up front versus later line, impact on overall survival, role of continued CDK 4/6 blockade, mechanism of clinical resistance, and treatment sequencing.

Summary The development of CDK 4/6 inhibitors has changed the therapeutic management of HR+ MBC. Additional research is needed to determine optimal treatment sequencing, understand mechanisms governing resistance, and develop novel therapeutic strategies to circumvent or overcome clinical resistance and further improve the outcomes of patients with MBC.

Keywords Metastatic breast cancer · Cyclin-dependent kinases 4 and 6 · Clinical trials · Resistance mechanisms · Palbociclib · Ribociclib · Abemaciclib

Introduction

Cell cycle abnormalities are common in cancer and have long been considered a potential therapeutic target. Cyclin-dependent kinases (CDKs) are critical regulatory enzymes governing cell cycle transitions and eventual cell division [1–4]. The tumor suppressor retinoblastoma (Rb) protein controls the key transition from G1 to the S phase [5]. Rb controls early cellular division by binding the E2F transcription factors to halt the G1/S transition, while inactivation of Rb permits cell division to proceed [6]. During G1, a variety of growth signals can result in cyclin D binding to either CDK4 or CDK 6 thereby causing phosphorylation of Rb and ultimate release of E2F and cell cycle progression [5]. The CDK4/6–Rb axis is important in a number of malignancies, particularly estrogen receptor-positive breast cancer, where estrogen has been shown to increase the rate of progression from the G1 to the S phase [7–9]. The binding of estrogen to ER-alpha drives cyclin D1 transcription, with activation of CDK4/6 and phosphorylation of Rb leading to subsequent cell cycling [10–12]. Selectively inhibiting CDK4/6 causes cell cycle arrest in the G1 phase, resulting in reduced cell viability and tumor response.

The development of CDK 4/6 inhibitors has changed the therapeutic management of hormone receptor-positive (HR+) metastatic breast cancer (MBC). Palbociclib, ribociclib, and abemaciclib are all orally active, highly selective reversible inhibitors of CDK4 and CDK6 approved by the Food and Drug Administration (FDA) for hormone receptor-positive (HR+) metastatic breast cancer in combination with specific endocrine therapies. This review will focus on the current use of CDK 4/6 inhibitors in HR+ MBC as well as current controversies and evolving areas of research.

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✉ Aditya Bardia
Bardia.Aditya@mgh.harvard.edu

[1] Massachusetts General Hospital Cancer Center, Harvard Medical School, Lawrence House 304, 10 North Grove St, Boston, MA 02114, USA

Pivotal Studies of CDK4/6 Inhibitors for HR+ MBC

Parallel drug discovery efforts led to the development of palbociclib, ribociclib, and abemaciclib. All three agents are

oral, potent, highly selective reversible inhibitors of CDK4 and CDK6 [13–15, 16•]. The phase III studies leading to FDA drug approvals for these agents are summarized in Table 1.

CDK 4/6 Inhibitors Plus Non-steroidal Aromatase Inhibitors

All three CDK 4/6 inhibitors have been studied in combination with a non-steroidal aromatase inhibitor in the first line and are FDA approved in this setting. For palbociclib, the phase III trial PALOMA-2 demonstrated a median PFS of 24.8 vs. 14.5 months, respectively, for patients treated with palbociclib or placebo in addition to letrozole (HR 0.58; $p < 0.001$) [17•]. The phase III MONALEESA-2 trial explored ribociclib in combination with letrozole as first-line therapy in women with HR+/HER2− MBC and showed the addition of ribociclib, as compared to placebo, improved PFS from 16 to 25.3 months (HR 0.57; $P < .001$) [16•]. In MONARCH 3, the addition of abemaciclib to letrozole or anastrozole significantly improved PFS (NR vs. 14.7 months; HR 0.54; $P < .001$) [18•].

CDK 4/6 Inhibitors Plus Fulvestrant

All three CDK 4/6 inhibitors have also been studied in combination with the selective estrogen-receptor degrader fulvestrant and are FDA approved in this setting. While the combination studies demonstrated significant improvement in PFS, there are some key study population differences between the phase III trials. For example, PALOMA-3 (fulvestrant with/without palbociclib) included both pre-menopausal

Table 1 Phase III studies of CDK 4/6 inhibitors in breast cancer

Study	CDK 4/6 inhibitor	Study population	Line of therapy	Sample size	Median PFS (months) vs. placebo
Trials in combination with NSAIs					
PALOMA-2 [17•]	Palbociclib	Postmenopausal women with HR+/HER2− ABC and no prior systemic treatment for ABC; (neo)adjuvant ET permitted if disease-free interval > 12 months from therapy completion	1st line	666	24.8 vs. 14.5 (HR 0.58; $P < .001$)
MONALEESA-2 [16•]	Ribociclib		1st line	668	25.3 vs. 16.0 (HR 0.57; $P < .001$)
MONARCH 3 [18•]	Abemaciclib		1st line	493	NR vs. 14.7 (HR 0.54; $P < .001$)
Trials in combination with fulvestrant					
MONALEESA-3 [19•]	Ribociclib	Postmenopausal women and men with HR+/HER2− ABC, 0–1 line of ET for ABC[b]	1st and 2nd line	726	20.5 vs. 12.8 (HR 0.60; $P < .001$)
MONARCH 2 [20•]	Abemaciclib	Women with HR+/HER2− ABC that had progressed during prior ET, any menopausal status, ≤ 1 ET, no prior CT for advanced disease[c]	2nd line	669	16.4 vs. 9.3 (HR 0.55; $P < .001$)
PALOMA-3 [21•, 22]	Palbociclib	Women with HR+/HER2− ABC that relapsed or progressed during ET, any menopausal status, ≤ 1 line of CT for advanced disease[a]	2nd line and plus	521	9.5 vs. 4.6 (HR 0.46; $P < .001$)
Trials in combination with tamoxifen or NSAI + Goserelin					
MONALEESA-7 [23•]	Ribociclib	Pre/perimenopausal women with HR+/HER2− ABC, no prior ET for advanced disease, ≤ 1 line of CT for advanced disease	1st line	672	23.8 vs. 13.0 (HR 0.55; $P < .001$)

Palbociclib dose was 125 mg daily orally and ribociclib dose was 600 mg daily orally on a 3-week on, 1-week off schedule in all studies. Abemaciclib final dose was 150 mg orally twice a day continuously

NSAI non-steroidal aromatase inhibitor, *ABC* advanced breast cancer, *ET* endocrine therapy, *CT* chemotherapy, *NR* not reached, *PFS* progression-free survival, *HR* hazard ratio

[a] Those with postmenopausal ABC must have had progression during prior aromatase inhibitor treatment; pre/perimenopausal patients must have had progression during prior ET. Pre/perimenopausal participants received goserelin for duration of study treatment starting ≥ 4 weeks prerandomization and continuing Q28D

[b] First line (i.e., treatment-naive for MBC): relapse > 12 months after (neo)adjuvant ET for localized breast cancer *or* de novo MBC with no prior ET. Second line/early relapsers: early relapse on or ≤ 12 months after (neo)adjuvant ET *or* relapse > 12 months after (neo)adjuvant ET with PD after 1 line ET for MBC *or* MBC with progression after 1 line ET for MBC

[c] Pre/perimenopausal participants received a gonadotropin-releasing hormone agonist

(goserelin added for premenopausal women) and postmenopausal women with HR+/HER2− MBC who had relapsed or progressed during prior endocrine treatment, and there was no limit on no of prior endocrine therapies (second-line and plus setting) [21•]. Overall, the addition of palbociclib resulted in an improvement in PFS from 4.6 months to 9.5 months (HR 0.46; $p < 0.001$). However, the MONARCH 2 (fulvestrant with/without abemaciclib) included women of any menopausal status (gonadotropin-releasing hormone agonist added for premenopausal women) with HR+/HER2− MBC who had progressed during prior endocrine therapy, but no more than one line (second-line setting) [20•]. Overall, the addition of abemaciclib resulted in an improvement in PFS from 9.3 to 16.4 months (HR 0.55; $P < .001$). The MONALEESA-3 trial explored fulvestrant with or without ribociclib in postmenopausal women and men with HR+/HER2− MBC who had received 0–1 lines of endocrine therapy for advanced disease, and thus included both first-line and second-line patients [19•]. Overall, the addition of ribociclib resulted in an improvement in PFS from 12.8 to 20.5 months (HR 0.60; $P < .001$).

Ribociclib in Pre-menopausal Women

Unlike the other phase III studies, MONALEESA-7 entirely focused on pre-menopausal women with HR+/HER2− MBC with no prior endocrine therapy for advanced disease, though permitted up to one line of chemotherapy for advanced disease. Patients were randomized to either tamoxifen/goserelin or NSAI/goserelin plus ribociclib or placebo. Overall, the addition of ribociclib improved PFS from 13 to 23.8 months (HR 0.55; $P < .001$) [23•].

Abemaciclib as Monotherapy

In the phase II single-arm study MONARCH 1, women with HR+/HER2− MBC with disease progression following both endocrine therapy and at least 1, but no more than 2, lines of chemotherapy in the metastatic setting, received abemaciclib as monotherapy [15]. The confirmed objective response rate was 19.7% and median PFS was 6.0 months [15]. Based on these results, abemaciclib is also approved as monotherapy at a dose of 200 mg twice a day.

Toxicities and Drug-Drug Interactions

The toxicity profile of the CDK4/6 inhibitors has been detailed in the clinical trials for each drug in the class (palbociclib–PALOMA; ribociclib–MONALEESA; abemaciclib–MONARCH) and post-marketing reports [16•, 17•, 18•, 20•, 21•, 22]. The CDK4/6 inhibitors are generally well tolerated and adverse events are manageable with dose modification and/or supportive care measures. There is some degree of overlap in the toxicity profile of each CDK4/6 inhibitor; however, there are unique features associated with each drug. The incidences of selective toxicities are outlined in Table 2.

Hematologic toxicities, primarily neutropenia, are common with the CDK4/6 inhibitors but are usually uncomplicated and managed with dose interruption and/or reduction. Severe events, including febrile neutropenia, are rare. Cytopenias are an on-target effect of CDK4/6 inhibitors due to the integral role of CDK6 in proliferation of hematologic precursors [24]. Unlike cytotoxic chemotherapy agents, which cause apoptosis, CDK4/6 inhibitors lead to a cytostatic effect on neutrophil precursors resulting in pharmacologic quiescence [25]; therefore, CDK4/6 inhibitor-induced neutropenia is rapidly reversible following withdrawal of the agent and myeloid growth factors (e.g., filgrastim) are not indicated in most cases. Palbociclib and ribociclib are dosed intermittently (e.g., 21 days on followed by 7 days off) to allow for recovery of hematologic cells. Abemaciclib demonstrates greater selectivity for CDK4 when compared to palbociclib and ribociclib, resulting in lower rates of hematologic toxicities, and thus can be dosed continuously [26]. Close monitoring of the complete blood count (CBC) is necessary during therapy with CDK4/6 inhibitors. Dose modification for hematologic toxicities is outlined in the prescribing information for each agent.

Common non-hematologic toxicities across the CDK4/6 inhibitor class include fatigue, nausea, vomiting, stomatitis, alopecia, rash, diarrhea, decreased appetite, and infections. These adverse effects are mild in the majority of patients and usually do not impact therapy. Each CDK4/6 inhibitor has the potential for fetal harm which requires women with reproductive potential to use effective contraception during and for at least 3 weeks after completion of therapy.

Ribociclib may result in hepatotoxicity which requires monitoring of liver function tests (LFTs) at baseline and during therapy. Median time to onset of severe hepatotoxicity (grade ≥ 3) was 85 days and median time to resolution to grade ≤ 2 was 22 days when used in combination with letrozole or fulvestrant. Dose interruption, reduction, or discontinuation of ribociclib may be required for hepatotoxicity. Ribociclib has been associated with reversible, concentration-dependent prolongation of the QT interval which appears to be unique to ribociclib out of the CDK4/6 inhibitor class. Cases of Torsades de Pointes were not reported in the clinical investigations of ribociclib. Monitoring of the QT interval should be done upon initiation and during therapy and ribociclib should not be used in patients who have or are at significant risk of developing QT prolongation (e.g., long QT syndrome, uncontrolled/significant cardiac disease, electrolyte abnormalities, concomitant use of medications with the potential for QT prolongation). Electrolytes should also be monitored in patients receiving ribociclib.

Abemaciclib results in higher rates of diarrhea and fatigue in comparison with palbociclib and ribociclib. Diarrhea

Table 2 Selected adverse events and laboratory abnormalities [16•, 18•, 21•]

Adverse event	Palbociclib/letrozole		Ribociclib/letrozole		Abemaciclib/NSAI	
	Any grade (%)	Grade 3–4 (%)	Any grade (%)	Grade 3–4 (%)	Any grade (%)	Grade 3–4 (%)
Neutropenia	80	66	75	60	41	22
Anemia	24	5	18	1	28	6
Thrombocytopenia	16	1	29[a]	1[a]	10	2
Nausea	35	< 1	52	2	39	< 1
Vomiting	16	1	29	4	28	1
Abdominal pain	NR	NR	11	1	35	2
Diarrhea	26	1	35	1	81	9
Decreased appetite	15	1	19	2	27	1
Cough	25	0	23	0	13	0
Stomatitis	30	1	12	< 1	15	< 1
Peripheral edema	NR	NR	12	0	12	0
Rash	18	1	17	1	11	1
Alopecia	33	N/A	33	0	16	0
Fatigue	37	2	37	2	46	3
AST increased	52	3	44	7	37	4
ALT increased	43	2	46	10	48	6
Creatinine increased	NR	NR	20	1	98	2
Infections	60	7	50	4	39	5

NSAI nonsteroidal aromatase inhibitor (letrozole or anastrozole), *NR* not reported, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *N/A* not applicable

[a] Reported as platelet count decreased

associated with abemaciclib is greatest during the first month of therapy. The median time to onset of diarrhea in MONARCH 2 and 3 was approximately 7 days and the median duration of grade 2–3 diarrhea was 6–11 days. Diarrhea may require dose interruption and/or reduction of abemaciclib. Antidiarrheals, such as loperamide, should be initiated at the onset of loose stools and patients should increase their oral fluid intake. Some clinical investigations of abemaciclib have utilized prophylactic loperamide though this is not specifically recommended in the prescribing information. Abemaciclib has also been associated with hepatotoxicity and LFT monitoring is recommended at baseline and during therapy. Median time to onset of severe hepatotoxicity (grade ≥ 3) in MONARCH 3 was approximately 60 days and median time to resolution to grade < 3 was approximately 14 days. Additionally, venous thromboembolic events (VTE) were reported in a greater proportion of patients receiving abemaciclib compared to placebo in MONARCH 2 and 3 (5% vs 0.9% and 5% vs 0.6%, respectively). Patients should be counseled regarding the risk and signs/symptoms of VTE when receiving abemaciclib. An increased level of serum creatinine (SCr) is common with abemaciclib as a result of inhibition of several tubular secretion transporters (OCT2, MATE1, and MATE2-K). This does not affect glomerular function and is not reflective of renal damage [15]. Alternative measures of renal function, such as blood urea nitrogen (BUN) or cystatin C, may be considered to monitor renal function in patients receiving abemaciclib.

CDK4/6 inhibitors are subject to interactions with other drugs primarily mediated by modification of the cytochrome P450 (CYP) pathway as palbociclib, ribociclib, and abemaciclib are all major substrates of the CYP3A4 enzyme. Therefore, concomitant use of strong CYP3A inhibitors/inducers with CDK4/6 inhibitors should be avoided whenever possible and alternate therapies should be considered. If a strong CYP3A inhibitor must be used along with palbociclib, ribociclib, or abemaciclib, dose reduction of the CDK4/6 inhibitor should be performed. Ketoconazole should be avoided with abemaciclib due to a significant increase in abemaciclib levels. Moderate CYP3A inhibitors/inducers should also be used with caution and dose modifications should be considered in accordance with each product's prescribing information, when applicable. Patients taking any CDK4/6 inhibitor should also avoid grapefruit and grapefruit juice due to the potential for increased drug exposure.

Palbociclib and ribociclib are weak and moderate inhibitors of CYP3A4, respectively. Therefore, concomitant use of CYP3A substrates with a narrow therapeutic index (e.g., cyclosporine, everolimus, fentanyl, tacrolimus) should be done cautiously and dose reductions of the CYP3A substrate may be

necessary. Drugs with the potential to prolong the QT interval (e.g., amiodarone, haloperidol, methadone, moxifloxacin, ondansetron, sotalol) should be avoided with ribociclib. Drug information resources should be utilized when assessing the potential for QT prolongation with specific drugs and the appropriateness of concomitant use with ribociclib. Notably, ribociclib is not indicated for use with tamoxifen despite its indication in pre/perimenopausal women. Abemaciclib inhibits P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). The clinical impact of abemaciclib on P-gp and/or BCRP substrates is not known, but concomitant use of sensitive substrates (e.g., digoxin) should be done cautiously.

Current Controversies

Should All Patients Receive a CDK 4/6 Inhibitor Up Front for HR+ MBC?

A number of patients with HR+/HER2− MBC have bone-only disease and there is certainly a subset of patients who experience stable disease for a prolonged duration of time with single agent endocrine therapy. However, the pivotal phase III trials of CDK 4/6 inhibitors in the first-line setting all show a near doubling of PFS with the addition of a CDK 4/6 inhibitor, with benefit seen across subgroups, including those with osseous only disease. Recent studies also suggest elderly patients benefit equally from CDK 4/6 inhibitors without unacceptable toxicity [27]. Until additional research further delineates which patients may do well in the first line without a CDK 4/6 inhibitor, it is reasonable to consider a CDK 4/6 inhibitor in combination with endocrine therapy for all first-line patients, if consistent with goals of care.

Another controversy is whether or not upfront endocrine therapy plus a CDK4/6 inhibitor or chemotherapy should be used for HR+ MBC patients with visceral disease and is an area of ongoing research in clinical trials. However, in the absence of visceral crisis, combination endocrine therapy and CDK 4/6 inhibitor therapy is reasonable for patients with visceral metastasis. Such patients were included in the pivotal trials and sub-analyses confirmed benefit.

Should an Aromatase Inhibitor or Fulvestrant Be Used as the Endocrine Backbone in Combination with a CDK 4/6 Inhibitor in the First-Line Setting?

Many clinicians use aromatase inhibitors in the first-line and fulvestrant in the second-line setting. The pivotal phase III trials of CDK 4/6 inhibitors in combination with NSAIs were first-line studies. However, the MONALEESA-3 trial looking at the addition of ribociclib to fulvestrant included first-line MBC patients, both those with de novo MBC and patients with relapse greater than a year after (neo)adjuvant endocrine therapy for localized breast cancer. First-line patients showed similar benefit as second-line patients, with a HR of 0.58. Notably, the phase III FALCON study comparing upfront anastrozole versus upfront fulvestrant for endocrine therapy-naïve HR+ MBC patients showed improved PFS with fulvestrant (16.6 vs. 13.8 months, HR 0.80, $P = 0.049$), with the most benefit seen in patients without visceral disease (22.3 vs. 13.8 months, HR 0.59) [28]. Based on the FALCON and MONALEESA-3 results, it would be very reasonable to consider fulvestrant in combination with a CDK 4/6 inhibitor for patients with de novo HR+ MBC, but whether it should be the preferred endocrine backbone for all patients is currently unclear.

Which CDK 4/6 Inhibitor Should Be Used?

While cross trial comparisons cannot be made, the hazard ratios of the first-line CDK 4/6 plus NSAI trials are remarkably similar and suggest the three agents likely have similar efficacy. The choice of CDK 4/6 inhibitor therefore largely comes down to considerations regarding adverse effects. As noted, palbociclib and ribociclib have more hematologic toxicities while abemaciclib has more gastrointestinal toxicity. The required monitoring of each agent varies somewhat as well. Comorbidities and concomitant medications are other considerations. For example, a patient with baseline issues with diarrhea from irritable bowel syndrome may favor palbociclib or ribociclib over abemaciclib. Given the potential for QTc prolongation with ribociclib, a patient who is already on potentially QTc-prolonging medications may do better with palbociclib or abemaciclib. Dose reductions, which are common with all three agents, are another factor to consider. Dose reductions were common in the pivotal trials and did not appear to compromise efficacy [29]. Dose adjustments are simplest with ribociclib as they can be done immediately rather than waiting for a new prescription to be processed. Cost and access are other important considerations. For example, some insurance plans may not include all three agents on their formulary. Lastly, clinician experience should not be overlooked. When all agents are a possibility, we would advocate patient-centered decision making with a discussion of the pros/cons of each agent.

Should CDK 4/6 Inhibitors Be Used After Progression?

To date, there are no data to support continuing CDK 4/6 inhibitor therapy beyond progression on CDK 4/6 therapy. This includes either switching to another CDK 4/6 inhibitor or switching the endocrine therapy partner and continuing a CDK 4/6 inhibitor. However, the concept is appealing, particularly when considering this approach has shown efficacy in other disease settings (for example, continuation of anti-HER2 therapy in HER2+ MBC), though toxicity and cost considerations need to be considered.

There are a number of trials exploring the continued use of CDK 4/6 inhibitors post-progression. For example, the phase II PACE study (NCT03147287) is looking at fulvestrant vs. fulvestrant + palbociclib ± avelumab. The TRINITI study (NCT02732119) is a phase I/II study exploring triplet therapy with ribociclib plus the mTOR inhibitor everolimus plus the steroidal AI exemestane following progression on prior CDK 4/6 inhibitor-based therapy. These studies will help answer this important question.

Is There an Overall Survival Improvement with CDK 4/6 Inhibitors?

Given the prognosis of HR+ MBC is measured in years and there are a number of therapies available, it is generally felt too difficult to demonstrate an overall survival benefit in this setting. There has been great interest in whether the impressive PFS benefits seen with CDK 4/6 inhibitors will translate to an overall survival (OS) benefit. However, the pivotal trials leading to approval of the CDK 4/6 inhibitors were powered for the primary endpoint of PFS, not OS.

Initial OS results from the phase II PALOMA-1 trial of letrozole ± palbociclib showed a statistically non-significant trend towards an improvement in OS a median OS of 37.5 months in the palbociclib/letrozole group compared to 34.5 months in the letrozole group (HR 0.897, 95% CI 0.623–1.294; $P = 0.281$) [30]. Similarly, the PALOMA-3 trial of fulvestrant ± palbociclib in the second line and beyond did not show an OS benefit overall (34.9 months vs. 28 months, HR 0.81, 95% CI 0.64–1.03, $P = 0.09$), but favored the palbociclib group with an absolute improvement of 6.9 months [31]. This trend was seen despite 16% of patients in the placebo-fulvestrant group receiving CDK 4/6 inhibitor treatment post-randomization. A significant OS benefit was seen in the subset of patients with sensitivity to previous endocrine therapy. Once survival data from the pivotal trials is more mature, a meta-analysis would be helpful to examine the OS question given limited power of the individual trials.

What Is the Optimal Second-Line Therapy in Patients Who Have Disease Progression on First-Line Therapy with CDK 4/6 Inhibitors?

It is unknown whether CDK 4/6 inhibitors could change tumor biology and how other therapies will fair in the post-CDK 4/6 setting. A standard approach after progression on a CDK 4/6 inhibitor + AI is single agent fulvestrant, but the PFS with fulvestrant in the post-CDK 4/6 setting is unclear. Similarly, the first approved targeted therapy in combination with endocrine therapy for HR+ MBC was the mTOR inhibitor everolimus in 2012 based on results of BOLERO-2 [32], and this combination remains a viable option for patients who have progressed on CDK 4/6 inhibitors [33]. Whether patients will continue to derive same degree of benefit with everolimus after disease progression on CDK 4/6 inhibitor is unclear and further research is needed to understand the response rates in this setting.

Emerging Insights into CDK4/6 Inhibitor Resistance

Despite the widespread use of CDK4/6 inhibitors in metastatic HR+ breast cancer, we have limited understanding of the genomic and molecular mechanisms governing resistance, though recent translational studies have begun to provide new insight. Preclinical development of the CDK4/6 inhibitors suggested preferential sensitivity in luminal HR+ cell lines with a greater degree of intrinsic resistance in basal and non-luminal cell lines [34]. Several studies have explored the development of acquired resistance after exposure of HR+ breast cancer cell lines to CDK4/6 inhibition in vitro.

Loss of RB1 expression occurred in HR+ breast cancer cells cultured to resistance in the presence of palbociclib as well as in a patient-derived xenograft model with prolonged exposure to ribociclib [35]. Parallel acquisition of cyclin E1 overexpression was also demonstrated in vitro, and these resistant cells could be resensitized to CDK4/6 therapy by targeting CDK2 activity [35]. Ectopic overexpression of CCNE1 and cyclin E2 (CCNE2) also provoked resistance to antiestrogen and palbociclib monotherapy in vitro, and these cyclin-overexpressing cells were more sensitive to CDK2-directed therapy [36]. HR+ breast cancer cells cultured to resistance in the presence of abemaciclib demonstrated increased levels of CDK6; the resulting cell lines were also cross-resistant to palbociclib and ribociclib [37]. These preclinical studies imply a central role for modulators of cell cycle progression in mediating resistance to CDK4/6 inhibitors, though clinical validation is needed.

To date, we have limited understanding of the molecular pathways governing clinical resistance in patient specimens, though several emerging studies are beginning to provide new insight. Acquired alterations in RB1 have been identified via targeted sequencing of circulating tumor DNA (ctDNA) in three patients (two with exposure to palbociclib and one with exposure to ribociclib) [38•]. Analysis of ctDNA from patients enrolled on the PALOMA-3 study also confirms unique enrichment of RB1 alterations in patients receiving the combination of palbociclib and fulvestrant, although this occurred in only 4.7% of the study population [38•]. PIK3CA and ESR1 mutations were also observed within the PALOMA-3 patient group; however, these alterations were well represented in both treatment arms [39•]. Whole exome sequencing of more than 50 patients with exposure to various CDK4/6 inhibitors demonstrated rare biallelic disruption in RB1, with a diverse array of mutational events contributing to protein malfunction (including homozygous deletion, splice alteration, point

mutation, and translocation) [40]. Besides RB1, amplification or mutation in AKT1, mutations in KRAS/HRAS/NRAS, amplification of CCNE2, amplification or mutation in FGFR2, mutation in ERBB2, and amplification of aurora kinase A (AURKA) have been implicated [40]. These preliminary observations provide new insights and highlight need for additional research to understand the molecular pathways driving clinical resistance to CDK 4/6 inhibitors.

Ongoing Trials and Future Directions

As reviewed, a number of studies are exploring key questions surrounding the use of CDK 4/6 inhibitors in the metastatic setting. Clinical trials such as the PACE and TRINITI studies among others will help answer whether or not patients will garner further benefit from CDK 4/6 inhibition post-progression. Combination studies with immunotherapy are also ongoing, with preclinical rationale suggesting CDK 4/6 exposure may enhance response to IO agents [41]. Ongoing translational studies will shed light onto the mechanisms of response and resistance to CDK 4/6 inhibition.

CDK 4/6 inhibitors are also being actively explored in the localized breast cancer setting, including neoadjuvant, adjuvant, and post-neoadjuvant studies. These are important studies given the majority of HR+ MBC presents as recurrent disease and late recurrences are common [42]. The phase II neoadjuvant NeoPalAna study demonstrated CDK 4/6 inhibitor results in enhanced antiproliferative activity HR+ early breast cancer, with 87% complete cell cycle arrest with palbociclib plus Anastrozole compared to 26% with anastrozole alone ($P < .001$) [43]. Similarly, the addition of abemaciclib to anastrozole was also shown to significantly reduce Ki67 compared to anastrozole alone [44]. Results from several other neoadjuvant trials, including the PALLET (NCT02296801) and FELINE (NCT02712723) studies, are anticipated. The phase III PENELOPE-B trial is studying the addition of palbociclib to standard endocrine therapy for HR+ patients with residual disease at high risk of relapse (NCT01864746). All three CDK 4/6 inhibitors are being studied in the standard adjuvant setting in phase III trials. The PALLAS trial is studying the addition of palbociclib to standard of care endocrine therapy in stage II/III HR+ breast cancer (NCT02513394), the monarchE trial is studying the adjuvant use of abemaciclib (NCT03155997), and the NATALEE trial is studying the adjuvant use of ribociclib (NCT03701334).

Conclusion

The approval of CDK 4/6 inhibitors for HR+ MBC has permanently changed the treatment paradigm of this disease. Palbociclib, ribociclib, and abemaciclib are each approved in combination with an aromatase inhibitor or fulvestrant, and abemaciclib is also approved as monotherapy for pre-treated patients. The most common adverse events associated with palbociclib and ribociclib are hematologic, particularly neutropenia, while gastrointestinal toxicities such as diarrhea are more common with abemaciclib. Most toxicities observed with CDK4/6 inhibitors are not complicated and are typically easily managed with standard supportive care and dose adjustments when indicated. There is much to be learned about the mechanisms of resistance and the role of CDK 4/6 inhibitors post-progression. A number of ongoing studies are exploring CDK 4/6 inhibitors in various disease settings for breast cancer, including adjuvant and neoadjuvant studies, and in combination with other targeted agents and immunotherapy in the advanced setting, as well as post-progression. Additional therapeutic strategies are needed to further improve the natural history of HR+ MBC.

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Compliance with Ethical Standards

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