Emerging data and future directions for CDK4/6 inhibitor treatment of patients with hormone receptor positive HER2-non-amplified metastatic breast cancer

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1 | INTRODUCTION

Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors in combination with endocrine therapy have been shown to improve progression free survival (PFS) in hormone receptor (HR) positive, human epidermal growth factor receptor 2 (HER2) non-amplified metastatic breast cancer (MBC) compared with endocrine therapy alone in both the first and second line treatment settings. This combination represents the current standard of care in an increasing number of countries, and has Food and Drug Administration (FDA) approval in the United States (US) and Pharmaceutical Benefits Scheme (PBS) approval in Australia.

Currently estrogen receptor (ER) positivity and HER2 negativity are the only biomarkers used to select patients with MBC for combination endocrine therapy and CDK4/6 inhibitor treatment. However, clinical trials suggest that a proportion of patients have de novo resistance to this treatment combination, and the vast majority of patients will inevitably develop resistance. The strategies to combat resistance to these combination treatments are not yet defined and represent the next major clinical challenge in ER-positive breast cancer. Further research is needed to identify predictive biomarkers of response and new therapeutic strategies for patients who progress on this combination therapy. In this review, we explore the mechanisms of resistance...
to CDK4/6 inhibitors and review potential biomarkers that may guide therapy selection. We also consider treatment options for patients who progress following treatment with a CDK4/6 inhibitor, the use of CDK4/6 inhibitors in the context of non-HR-positive/HER2-non-amplified breast cancer, and combinations with therapies other than endocrine therapy.

2 | BIOMARKERS OF RESPONSE TO CDK4/6 INHIBITORS

CDK4/6 inhibitors are effective in both endocrine-sensitive and endocrine-resistant breast cancer. This has been demonstrated in preclinical studies, and then subsequently in the seminal clinical trials, which have included treatment-naïve patients as well as patients who had relapsed or progressed on endocrine therapy. A core reason underlying the efficacy of CDK4/6 inhibitors is their ability to overcome some of the mechanisms that mediate endocrine-resistance, although the eventual progression of disease in the majority of patients treated points to the existence or development of overlapping resistance mechanisms.

Potential predictive biomarkers of response have been evaluated for CDK4/6 inhibitors. Other than ER and retinoblastoma (Rb) positivity, the results have been disappointing in general with none having been clinically validated in patients with HR-positive, HER2-non-amplified breast cancer to date. Dysregulation of the cyclin-CDK-Rb axis by upregulation of cyclin-CDK activity and/or abrogation of suppressors are features of many tumor types, including ER-positive breast cancer. In this cancer, growth is driven by estradiol, which consequently drives cellular proliferation, partly through the ER-positive breast cancer. In this cancer, growth is driven by estradiol, abrogation of suppressors are features of many tumor types, including cyclin-CDK-Rb axis by upregulation of cyclin-CDK activity and/or HER2-non-amplified breast cancer to date. Dysregulation of the none having been clinically validated in patients with HR-positive, HER2-non-amplified breast cancer to date. Dysregulation of the cyclin-CDK-Rb pathway did not correlate with greater benefit from palbociclib.

One study in patients with HR-positive MBC evaluated the association between response to CDK4/6 inhibitors in second line use and beyond and PIK3CA and TP53 mutations in metastatic tumor DNA. No significant difference in PFS was seen among patients with PIK3CA mutations (7.1 vs. 9.7 months; \( P = 0.28 \)) or with any alteration in the PI3K/Akt/mTOR pathway (8.2 vs. 9.3 months; \( P = 0.40 \)). Patients who had TP53 mutations or complex tumor genomics had a trend toward shorter PFS, however statistical significance was not reached possibly due to the small number of patients in this subgroup.

A study of baseline tumor tissue samples from patients in the PALOMA-3 trial found that patients with low tumor cyclin E expression experienced a greater benefit from palbociclib compared to patients with high expression (median PFS 14.1 vs. 7.6 months, \( P = 0.0024 \)). However, other cell cycle genes did not show any interaction as a biomarker, with all subgroups deriving similar benefit from palbociclib compared to placebo.

Studies of baseline circulating tumor DNA (ctDNA) and tumor tissues from the PALOMA trials found no link between response to palbociclib and PIK3CA mutational status. In contrast, when sequential plasma samples at baseline and day 15 were studied in a subset of patients in the PALOMA-3 study, a relative decrease in PIK3CA ctDNA strongly predicted for a PFS improvement with palbociclib plus fulvestrant (HR 3.94; 95% CI 1.61–9.64; \( P = 0.0013 \)). In this study, ESR1 mutations (a mutation in gene coding for the ERa) were not predictive of long term PFS improvement. Similar correlative studies performed in the MONALEESA-2 clinical trial demonstrated that the addition of ribociclib to letrozole resulted in an improved PFS irrespective of the status of baseline ctDNA biomarkers (RTK or ZNF703/FGFR1 alterations).
In a small study, the longitudinal mutational landscape was evaluated in the ctDNA of 15 patients who progressed during treatment with palbociclib plus endocrine therapy. There was stable and persistent incidence of PIK3CA, TP53 and ESR1 mutations before treatment and at progression. In addition, there was also a low but measurable incidence of new mutations found in other genes, and an increased rate of MYC gene amplification that may be related to drivers of CDK4/6 inhibitor-resistance.16

3 | NOVEL THERAPEUTIC COMBINATIONS WITH CDK4/6 INHIBITORS IN ENDOCRINE-RESISTANT BREAST CANCER

While CDK4/6 inhibitors have been developed for clinical use primarily in the context of combination with endocrine therapy, a number of rational combinations with other classes of drugs can also be considered in the setting of progression on endocrine-based therapy based upon their ability to overcome endocrine-resistance. These preclinical studies provide the rationale for the further clinical development of novel therapeutic combinations with CDK4/6 inhibitors. Details of ongoing clinical trials of novel combinations with CDK4/6 inhibitors are listed in Table 1.

First, there is a growing number of endocrine therapies being developed that are potentially more effective than tamoxifen, aromatase inhibitors and fulvestrant. These new endocrine therapies would make logical partners with CDK4/6 inhibitors. Next generation selective ER degraders (SERDs) currently in clinical development have been shown to be more potent than their prototype fulvestrant with activity in endocrine-resistance and ESR1 mutations.17 Furthermore, many of these are orally bioavailable in contrast to fulvestrant, which is administered as an injection, increasing patient convenience. One such example is elacestrant (RAD1901), which has enhanced the efficacy of both palbociclib and abemaciclib in vitro.18 Other SERDs that are currently being evaluated in clinical trials in combination with CDK4/6 inhibitors include LSZ102 in combination with ribociclib (NCT02734615; Table 1), and GDC 9545 in combination with palbociclib (NCT03332779; Table 1).

Other potent ER-directed agents in development include the ER coregulatory binding modulator EtiRaRx-11 (ERX-11), which has demonstrated efficacy in blocking ER and ER-coregulator mediated oncogenic signaling, and inhibited proliferation of both endocrine-sensitive and -resistant breast cancer cells. Co-treatment of ERX-11 with palbociclib showed synergistic activity preclinically in vitro and in vivo models.19 Another group of compounds with a unique ER antagonist activity in clinical development are the selective estrogen receptor covalent antagonists (SERCAs), which include H3B-5942. H3B-5942 covalently inactivates both wild-type and mutant ERα by targeting Cys530 and enforcing a unique antagonist conformation resulting in antitumor activity in vitro and in vivo. Additionally, the efficacy of H3B-5942 was further improved in combination with palbociclib.20

The PI3Ks signaling pathway is a major alternative signaling pathway that is upregulated in endocrine-resistance (Figure 1). Everolimus, a mammalian target of rapamycin (mTOR) inhibitor, in combination with exemestane, has demonstrated prolonged median PFS compared with exemestane alone in patients who had progressed on nonsteroidal aromatase inhibitors (NSAIs) in the BOLERO-2 trial (7.8 vs. 3.2 months; HR 0.45; 95% CI 0.38–0.54)21 and has been FDA and PBS approved for this indication. Similarly, everolimus extended PFS when added to fulvestrant (12.2 vs. 7.6 months; HR 0.64; 95% CI 0.45–0.91).22 Preclinical studies with triplet combinations of PI3K/mTOR inhibitors, CDK4/6 inhibitors and endocrine therapies have been promising, forming the basis of a number of phase I clinical studies currently underway (Table 1).23–27 Initial results suggest that these combinations are feasible with manageable toxicity and showed encouraging signs of clinical activity including in some patients with prior exposure to PI3K/AKT/mTOR or CDK4/6 inhibitors.25–27 One study found that duration of treatment was longer in patients with cyclin D amplification, possibly due to the inclusion of a CDK4/6 inhibitor. These results suggest that triplet therapy might be beneficial for patients who progress on doublet therapy or who have cyclin D amplification.26 Entinostat is an oral, class 1, selective histone deacetylase (HDAC) inhibitor, which has been shown to inhibit ER-positive breast cancer cells and restore endocrine sensitivity as a result of the downregulation of estrogen-independent growth factor signaling pathways.28 The phase II trial of exemestane with or without entinostat demonstrated an improvement in median PFS (4.3 vs. 2.3 months; HR 0.73, 95% CI 0.50–1.07) and median overall survival (OS) (28.1 vs. 19.8 months; HR 0.59, 95% CI 0.36–0.97).29 Consequently, a phase 3 trial of this combination is currently underway in patients with advanced ER-positive breast cancer (NCT02115282). The combination of entinostat and palbociclib has demonstrated synergistic activity in vitro and thus also warrants further exploration.30

Other signaling pathways that are upregulated in the context of endocrine-resistance include the fibroblast growth factor receptor 1 (FGFR1) and insulin-like growth factor 1 (IGF-1) signaling pathway. FGFR1 amplification occurs in approximately 10% of ER-positive human breast cancers, and is associated with early recurrence after adjuvant endocrine therapy.31 The ER signaling pathway remains active in ER-positive, FGFR1-amplified breast cancer models in estrogen-deprived conditions.32 These findings have led to the initiation of a phase 2 trial of lucitanib, a vascular endothelial growth factor (VEGF), platelet-derived growth factor receptor (PDGFR) and FGFR inhibitor in patients with ER-positive metastatic breast cancer (NCT02053636). IGF ligand-dependent signaling via the IGF receptor results in upregulation of cyclin D1, and subsequent progression through the cell cycle,33 and the combination of palbociclib with IGF-1 receptor (IGF-1R) inhibitory antibody has demonstrated promising in vitro activity in preclinical ER-positive breast cancer cells.34 A phase I clinical trial (NCT03099174; Table 1) of an IGF-1 ligand-neutralizing antibody, xentuzumab, in combination with abemaciclib, with and without endocrine therapy, is currently underway.

Finally, CDK4/6 inhibitors have also been shown to increase tumor immunogenicity through the activation of tumor cell expression of endogenous retroviral elements and enhancement of tumor antigen presentation, as well as by the suppression of regulatory T cell (Treg) proliferation.35 This seminal finding has provided a rationale for new combination regimens comprising CDK4/6 inhibitors and...
TABLE 1  Current accruing clinical trials for the treatment of hormone receptor positive, HER2-non-amplified metastatic breast cancer

<table>
<thead>
<tr>
<th>Study Title</th>
<th>Agents</th>
<th>Therapy class</th>
<th>Study status</th>
<th>Estimated completion date</th>
<th>Clinicaltrials.gov identifier</th>
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<td><strong>Novel therapeutic combinations with CDK4/6 inhibitors</strong></td>
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<tr>
<td>A phase I/ib study of LSZ102 (LSZ102+ ribociclib or LSZ102 + alpelisib) in patients with advanced ER+ breast cancer who have progressed after endocrine therapy</td>
<td>Ribociclib Alpelisib LSZ102</td>
<td>CDK4/6i PI3K/mTORi SERD</td>
<td>Recruiting</td>
<td>February 2018</td>
<td>NCT02734615</td>
<td>74</td>
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<tr>
<td>A phase Ia/Ib, study of GDC 9545 alone or in combination with Palbociclib and/or LHRH Agonist in patients with advanced ER+ breast cancer</td>
<td>Palbociclib GDC 9545 LHRH agonist</td>
<td>CDK4/6i SERD LHRH agonist</td>
<td>Recruiting</td>
<td>July 2021</td>
<td>NCT03332797</td>
<td>N/A</td>
</tr>
<tr>
<td>A phase Ib study of Gedatolisib in combination with Palbociclib and either Letrozole or Fulvestrant in women with advanced ER + breast cancer</td>
<td>Palbociclib Gedatolisib Fulvestrant or Letrozole</td>
<td>CDK4/6i PI3K/mTORi SERD NSAI</td>
<td>Recruiting</td>
<td>November 2019</td>
<td>NCT02684032</td>
<td>25</td>
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<tr>
<td>A phase Ib study of Ribociclib in combination with Everolimus and Exemestane in postmenopausal women with advanced ER+ breast cancer</td>
<td>Ribociclib Everolimus Exemestane</td>
<td>CDK4/6i PI3K/mTORi SAI</td>
<td>Active, not recruiting</td>
<td>September 2018</td>
<td>NCT01857193</td>
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<tr>
<td>A phase Ib/Ii, study of the combination of Ribociclib and Alpelisib with letrozole in patients with advanced ER+ breast cancer</td>
<td>Ribociclib Alpelisib Letrozole</td>
<td>CDK4/6i PI3K/mTORi NSAI</td>
<td>Recruiting</td>
<td>March 2019</td>
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<td>A phase Ib study of Xentuzumab and Abemaciclib in patients with advanced solid tumors and in combination with endocrine therapy in patients with advanced ER+ breast cancer</td>
<td>Abemaciclib Xentuzumab Letrozole Anastrozole Fulvestrant</td>
<td>CDK4/6i IGF antibody NSAI SERD</td>
<td>Recruiting</td>
<td>December 2018</td>
<td>NCT03099174</td>
<td>33</td>
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<tr>
<td>A phase Ib study of Abemaciclib in combination with Pembrolizumab for patients with advanced non-small cell lung cancer or ER+ breast cancer</td>
<td>Abemaciclib Pembrolizumab</td>
<td>CDK4/6i PD-L1 antibody</td>
<td>Recruiting</td>
<td>May 2019</td>
<td>NCT02779751</td>
<td>36</td>
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<td>A phase II study of the combination of Pembrolizumab, Letrozole and Palbociclib in postmenopausal patients with newly diagnosed advanced ER+ breast cancer</td>
<td>Palbociclib Pembrolizumab Letrozole</td>
<td>CDK4/6i PD-L1 antibody NSAI</td>
<td>Recruiting</td>
<td>September 2018</td>
<td>NCT02778685</td>
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<td><strong>Potential treatment strategies to overcome resistance to CDK4/6 inhibitors</strong></td>
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<td>A phase Ib trial of Fulvestrant, Palbociclib, and Erdafitinib in advanced ER+/HER2-/FGFR-amplified breast cancer</td>
<td>Palbociclib Erdafitinib Fulvestrant</td>
<td>CDK4/6i FGFR inhibitor SAI</td>
<td>Recruiting</td>
<td>September 2020</td>
<td>NCT03238196</td>
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<td><strong>Treatment of HR-positive HER2-non-amplified MBC following progression with a CDK4/6 inhibitor</strong></td>
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<td>A phase II study of Fulvestrant, Palbociclib, and Avelumab for endocrine pre-treated ER+ advanced breast cancer</td>
<td>Palbociclib Fulvestrant Avelumab</td>
<td>CDK4/6i SERD PD-L1 antibody</td>
<td>Recruiting</td>
<td>December 2020</td>
<td>NCT03147287</td>
<td>57</td>
</tr>
<tr>
<td>A phase II, study of Alpelisib Plus Fulvestrant or Letrozole in patients with PIK3CA mutant, ER+ advanced breast cancer, who have progressed on or after CDK4/6i treatment</td>
<td>Alpelisib Fulvestrant or Letrozole</td>
<td>PI3K/mTORi SERD NSAI</td>
<td>Recruiting</td>
<td>February 2020</td>
<td>NCT03056755</td>
<td>58</td>
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<tr>
<td>A phase II study of Fulvestrant with or without Ribociclib after progression on anti-estrogen therapy plus CDK4/6i in patients with advanced ER+ breast cancer</td>
<td>Ribociclib Fulvestrant</td>
<td>CDK4/6i SERD</td>
<td>Recruiting</td>
<td>May 2019</td>
<td>NCT02632045</td>
<td>59</td>
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<tr>
<td>A phase I/Ii, study of Ribociclib in combination with Everolimus + Exemestane in the treatment of men and postmenopausal women with ER+ advanced breast cancer following progression on a CDK 4/6i</td>
<td>Ribociclib Everolimus Exemestane</td>
<td>CDK4/6i PI3K/mTORi SAI</td>
<td>Recruiting</td>
<td>May 2018</td>
<td>NCT02732119</td>
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<td><strong>Patients with CNS metastases</strong></td>
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<tr>
<td>A phase II study of Abemaciclib in patients with brain metastases secondary to ER+ breast cancer, non-small cell lung cancer, or melanoma</td>
<td>Abemaciclib</td>
<td>CDK4/6i</td>
<td>Recruiting</td>
<td>November 2018</td>
<td>NCT02308020</td>
<td>64</td>
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<tr>
<td>A phase II study of Palbociclib in patients with metastatic HER2-positive breast cancer with brain metastasis</td>
<td>Palbociclib</td>
<td>CDK4/6i</td>
<td>Recruiting</td>
<td>November 2019</td>
<td>NCT02774681</td>
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(Continues)
immunotherapies. Pembrolizumab, an anti-PD-1 antibody, is currently being evaluated in combination with abemaciclib in a phase II clinical trial (NCT02779751; Table 1). Pembrolizumab is also being evaluated in a phase II study (NCT02778685; Table 1) in postmenopausal patients who have achieved stable disease during treatment with letrozole and palbociclib.

4 | RESISTANCE TO CDK4/6 INHIBITORS

Many of the preclinical studies to date have evaluated single agent resistance to CDK4/6 inhibitors, in contrast to the clinical context in which resistance occurs in combination with endocrine therapy. An important consideration is how the ER and CDK4/6 directed therapy resistance mechanisms overlap. CDK4/6 inhibition acts directly downstream of endocrine therapy and it is therefore inevitable that some mechanisms of resistance will be common to both types of treatments.

Mechanisms of endocrine-resistance have been well described, including upregulation of alternate signaling pathways, genomic and epigenetic aberrations. Rb is maintained in the vast majority of ER-positive breast cancer, and in most cases functional Rb protein is retained through the development of endocrine-resistance, rendering these tumors amenable to CDK4/6 inhibition. In addition, the incidence of Rb gene deletion/mutation is very rare (3.9%) in ER-positive breast cancer. While there have been preclinical studies demonstrating that Rb loss is a mechanism for resistance to CDK4/6 inhibition, the jury is still out as to whether Rb is a predictive biomarker for CDK4/6 inhibitor response as there are studies in other cancer types to suggest that functional Rb is not an absolute requirement for CDK4/6 inhibitors to be effective.

Other proposed mechanisms of resistance to CDK4/6 inhibitors include the upregulation of CDK4 or CDK6. In one study, CDK4 T172-phosphorylation was found to correlate with sensitivity to palbociclib, and an 11-gene expression signature that predicted CDK4 modification profiles of breast tumors and cell lines correlated with palbociclib response. Low levels of CDK6 expression have been shown to be positively correlated with the efficacy of ribociclib in endocrine-resistant breast cancer cell lines. CDK6 has also been shown to play a role in mediating endocrine-resistance, highlighting the potential overlap in mechanisms of resistance to endocrine therapy and CDK4/6 inhibitors. Increased expression of CDK6 have been described in fulvestrant-resistant breast cancer cell lines. Furthermore, high CDK6 levels in

### TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Study Title</th>
<th>Agents</th>
<th>Therapy class</th>
<th>Study status</th>
<th>Estimated completion date</th>
<th>Clinicaltrials.gov identifier</th>
<th>Ref.</th>
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<tr>
<td>A phase II study of Abemaciclib plus Trastuzumab with or without Fulvestrant vs chemotherapy of physician’s choice Plus trastuzumab in patients with HR+, HER2+ advanced breast cancer</td>
<td>Abemaciclib Trastuzumab Fulvestrant</td>
<td>CDK4/6i HER2 antibody SERD</td>
<td>Active, not recruiting</td>
<td>August 2018</td>
<td>NCT02675231</td>
<td>69</td>
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<td>A phase II study of Ribociclib, in combination with Trastuzumab or T-DM1 for advanced HER2+ breast cancer</td>
<td>Ribociclib Trastuzumab T-DM1 Fulvestrant</td>
<td>CDK4/6i HER2 antibody HER2 antibody-chemotherapy conjugate SERD</td>
<td>Recruiting</td>
<td>February 2019</td>
<td>NCT02657343</td>
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<td>A phase II study Tucatinib in combination with Palbociclib and Letrozole in subjects with advanced ER+HER2+ breast cancer</td>
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<td>CDK4/6i TKI NSAI</td>
<td>Recruiting</td>
<td>October 2018</td>
<td>NCT03054363</td>
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<tr>
<td>A phase III study of Palbociclib + Anti-HER2 therapy + endocrine therapy vs. Anti-HER2 therapy + endocrine therapy after induction treatment for advanced ER+HER2+ breast cancer</td>
<td>Palbociclib Trastuzumab Pertuzumab Letrozole or Anastrozole or Exemestane or Fulvestrant</td>
<td>CDK4/6i HER2 antibody NSAI NSAI NSAI SERD</td>
<td>Recruiting</td>
<td>October 2020</td>
<td>NCT02947685</td>
<td>72</td>
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<tr>
<td>A phase III study of chemo vs endocrine therapy in combination with dual HER2-targeted therapy in patients with advanced ER+HER2+ breast cancer</td>
<td>Ribociclib Trastuzumab Pertuzumab Letrozole or Anastrozole or Exemestane or Fulvestrant or Tamoxifen Paclitaxel or Vinorelbine or Docetaxel</td>
<td>CDK4/6i HER2 antibody NSAI NSAI NSAI SERD SERM chemo</td>
<td>Recruiting</td>
<td>September 2021</td>
<td>NCT02344472</td>
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CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; CNS, central nervous system; PI3K/mTOR, phosphatidylinositol-3 kinase/mammanian target of rapamycin inhibitor; SERD, selective estrogen receptor degrader; LHRH agonist, luteinizing hormone-releasing hormone agonist; NSAI, non-steroidal aromatase inhibitor; SAI, steroidal aromatase inhibitor; IGF, insulin growth factor; PD-L1, programmed death ligand 1; FGFR, fibroblast growth factor receptor; TKI, tyrosine kinase inhibitor; SERM, selective estrogen receptor modulator.
metastatic tumor tissue from two independent cohorts of breast cancer patients treated with fulvestrant (n = 45 and 46) correlated significantly with shorter PFS in patients treated with fulvestrant (median time to progression 2.5 vs. 8.2 months, \( P = 0.0006 \) and 3.4 vs. 8.9 months, \( P = 0.018 \)). Treatment with palbociclib resulted in alteration of Rb protein phosphorylation and re-sensitization of the cells to fulvestrant in preclinical models.\(^{48}\)

5 | POTENTIAL TREATMENT STRATEGIES TO OVERCOME RESISTANCE TO CDK4/6 INHIBITORS

In addition to playing a role in endocrine-resistance, FGFR1 amplification has also been reported to induce resistance to fulvestrant and CDK4/6 inhibitor combinations in gain-of-function kinase library screens in cell lines.\(^{49}\) The resistance phenotype was abrogated with the FGFR tyrosine kinase inhibitor lucitanib. Furthermore, in the MONALEESA-2 study, FGFR1 amplification in ctDNA was shown to correlate with early progression on ribociclib.\(^{15}\) The data suggest that FGFR signaling is a mechanism of resistance to endocrine therapies with or without CDK4/6 inhibitors, with overexpression of cyclin D1 induced by both FGFR signaling and ER transcription.\(^{49}\) Additionally, two activating FGFR2 mutations (M538I and N550K) have been identified in patients with endocrine-resistant ER-positive MBC, which are associated with resistance to fulvestrant, palbociclib and the combination of the two agents.\(^{50}\) Building on these findings, a phase Ib study has been initiated to evaluate fulvestrant, palbociclib and erdafitinib (an FGFR inhibitor) in patients with endocrine-resistant ER-positive, HER2-non-amplified and FGFR1-4 amplified MBC (NCT03238196; Table 1).

In another study using ribociclib-resistant breast cancer cells, dinaciclib, a pan-CDK inhibitor and GSK2334470, a 3-phosphoinositide dependent protein kinase 1 (PDK1) inhibitor was found to re-sensitize the resistant cells to CDK4/6 inhibitors.\(^{51}\) Ribociclib plus GSK2334470 completely abrogated components of the cell cycle, including pRb, p56, pRSK2, pCDK2, cyclin A and cyclin E, making this combination another potential strategy to overcome CDK4/6 inhibitor resistance.

Finally, higher activator protein 1 (AP-1) transcriptional activity has been demonstrated in dually tamoxifen and palbociclib-resistant cell lines.\(^{52}\) The combination of palbociclib and AP-1 blockade was effective in inhibiting cell growth and reducing pRb and CDK2 levels compared to either agent alone.

6 | TREATMENT OF HR-POSITIVE, HER2-NON-AMPLIFIED MBC FOLLOWING PROGRESSION ON A CDK4/6 INHIBITOR

This clinical scenario will increase in frequency as CDK4/6 inhibitors become established as the standard of care for patients with advanced ER-positive breast cancer in combination with endocrine therapy. There are currently no treatment guidelines as to how to manage patients who progress on CDK4/6 inhibitors, even though CDK4/6 inhibitor use is most often confined to the first and second line settings. Several studies have investigated the follow-up treatment of patients after participation in clinical trials with a CDK4/6 inhibitor. In patients studied for 9 months immediately after participation in the PALOMA-3 study, the median time to subsequent chemotherapy was significantly longer for the palbociclib arm compared to the placebo arm (252 vs. 132 days), and fewer patients in the palbociclib arm discontinued next-line treatment (33% vs. 46%), suggesting that palbociclib did not adversely affect the efficacy of subsequent treatments.\(^{53}\) Similar trends were observed for post-progression treatment of patients receiving the next line of therapy immediately following participation in the PALOMA-1 trial.\(^{54}\) In the MONALEESA-2 study, ribociclib plus letrozole also prolonged the time to next treatment compared to letrozole alone (24.2 vs. 16.7 months).\(^{55}\) Fewer patients treated with the combination received subsequent chemotherapy compared with letrozole alone (15.8% vs. 22.4%).

In contrast, there are case studies of rapid secondary disease progression during subsequent lines of therapy following cessation of CDK4/6 inhibitors.\(^{56}\) This observation has not been reported in larger CDK4/6 inhibitor clinical trials.

Table 1 outlines several phase II clinical trials that are ongoing to investigate the treatment of HR-positive, HER2-non-amplified MBC with endocrine therapy, CDK4/6 inhibitors or PI3K/mTOR inhibitors following progression on a CDK4/6 inhibitor.\(^{57-60}\)

7 | OTHER CLINICAL SCENARIOS IN WHICH CDK4/6 INHIBITORS MAY PLAY A ROLE IN BREAST CANCER

7.1 | Patients with central nervous system (CNS) metastases

A lower percentage of patients with HR-positive, HER2-non-amplified MBC develop brain metastases when compared with patients with triple negative breast cancer or HER2-amplified MBC.\(^{61}\) However, owing to the high incidence of the HR-positive, HER2-non-amplified breast cancer sub-type, cases are still relatively common. There are no specific regulatory approved systemic agents for the treatment of HR-positive breast cancer brain metastases, and so this remains an unmet medical need. Standard local treatment options include surgery, stereotactic radiosurgery (SRS) and/or whole brain radiation therapy (WBRT).\(^{61}\)

There is preliminary evidence and preclinical data that ribociclib, abemaciclib and palbociclib cross the brain–blood barrier,\(^{62,63}\) with data suggesting that abemaciclib may be more efficient in crossing the blood–brain barrier compared to palbociclib.\(^{62}\) Several clinical trials are ongoing to evaluate CDK4/6 inhibitors in patients with brain metastases (Table 1).\(^{64,65}\)

7.2 | HR-positive, HER2-amplified MBC

There have been results from three phase II clinical studies investigating the treatment of HER2-amplified MBC with palbociclib. PATRICIA
assessed the efficacy of palbociclib and trastuzumab in advanced HER2-amplified breast cancer (patients had received two to four prior lines of anti-HER2 based therapies). Patients with luminal disease showed a statistically significantly longer median PFS than patients with non-luminal disease (10.37 vs. 3.53 months, \( P = 0.023 \)) and it was concluded that patients with non-luminal disease might not derive a large benefit from this treatment strategy.66 Another clinical trial investigated the addition of palbociclib with or without trastuzumab in patients with Rb-positive advanced cancer, including a sub-cohort of patients with HER2-amplified disease. Of the 10 patients with HER2-amplified disease receiving palbociclib plus trastuzumab, a response rate of 30% and a PFS of 6.7 months (95% CI 1.6–17.8) was observed. It was concluded that further investigation into this combination was warranted.67 Neoadjuvant triplet therapy with trastuzumab, pertuzumab and palbociclib for women with non-metastatic HER2-amplified breast cancer was also investigated (NCT02530424). The primary endpoint was characterization of Ki67 changes from baseline before therapy, at 2 weeks and at surgery. This triplet targeting resulted in a significant and rapid decrease of Ki67 that was of larger magnitude after 2 weeks than at surgery irrespective of the recorded objective clinical response. It was suggested that tolerability and clinical response merit further clinical testing and additional molecular characterization of this chemotherapy-free approach.68

There are several ongoing clinical trials investigating CDK4/6 inhibitor combinations in patients with advanced HR-positive, HER2-amplified breast cancer (Table 1). There are two studies evaluating the combination of a CDK4/6 inhibitor with trastuzumab with or without fulvestrant.69,70 A phase Ib/II study will test the combination of the novel oral HER2 small molecule inhibitor tucatinib with palbociclib and letrozole as a potential non-chemotherapy based regimen.71 PATINA (NCT02947685; Table 1) is a phase III registration trial investigating the addition of palbociclib to dual HER2-directed (pertuzumab and trastuzumab) and endocrine therapy to determine if the addition of a CDK4/6 inhibitor will prolong PFS compared to dual HER2-directed and endocrine therapy.72 Similarly, the DETECT V study (NCT02344472; Table 1) is comparing the safety and efficacy of dual HER2-directed (pertuzumab and trastuzumab) and endocrine therapy with ribociclib.73

Several clinical trials are investigating novel therapeutic combinations with CDK4/6 inhibitors in endocrine-resistant breast cancer. These include the combination of CDK4/6 inhibitors with next generation SERDs and new classes of endocrine therapies, HDAC inhibitors, IGFR and FGFR inhibitors and immunotherapies.29,33,74 Triplet combinations of CDK4/6 inhibitors, PI3K/mTOR inhibitors and endocrine therapies, and CDK4/6 inhibitors, HER2-directed antibodies and endocrine therapies are also being explored.23–27,37 There are also concerted efforts to identify potential treatment strategies to overcome CDK4/6 inhibitor resistance, including the use of FGFR, PIK3CA, PDK1 and AP-1 inhibitors.49,51,52

There are several ongoing clinical trials to investigate treatment of patients with HR-positive, HER2-non-amplified MBC following progression on a CDK4/6 inhibitor and endocrine therapy, a major emerging clinical challenge where there is currently little data and no guidelines.

Given the observed benefit of CDK4/6 inhibitors in combination with endocrine therapy for patients with HR-positive, HER2-non-amplified MBC, large adjuvant studies are now underway to investigate the combination in HR-positive, HER2-non-amplified early breast cancer.75–77

CDK4/6 inhibitors in combination with endocrine therapy represent a significant development in the treatment of HR-positive, HER2-non-amplified MBC. However, identifying therapeutic strategies to follow this treatment is of immediate clinical importance since the vast majority of patients will inevitably progress on this combination.

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F.B. has served as an investigator on CDK inhibitor trials, and on advisory boards for Pfizer, Novartis, Eli Lilly, AstraZeneca and Roche. N.W. has been an investigator on CDK inhibitor trials, owns stock in CSL, has participated in advisory boards for Roche and Novartis and has provided editorial support to Pfizer. R.H. has participated in advisory boards for Novartis, Merck Sharp and Dohme, AstraZeneca, Roche, Bristol Myer Squibb and Pfizer and has received speaker honoraria from Novartis, Merck Sharp and Dohme, AstraZeneca, Roche, Bristol Myer Squibb and Boehringer Ingelheim. J.B. has participated in advisory boards for Novartis, Merck Sharp and Dohme, AstraZeneca, Roche, Bristol Myer Squibb and Pfizer and has received speaker honoraria from Novartis, Amgen and Genomic Health. A.R. has been an investigator on CDK inhibitor trials, and participated on advisory boards for Pfizer, Novartis, Roche and Eisai. E.L. has received research funding from Novartis and Bayer Pharmaceuticals, and has participated in advisory boards for Pfizer, Novartis, Roche, Eisai and Lilly. N.M.C. has been an investigator on CDK inhibitor trials and participated in advisory boards for Pfizer, Novartis, Roche and Astra Zeneca.
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