Inhibitors targeting CDK4/6, PARP and PI3K in breast cancer: a review

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Abstract: Breast cancer is the global leading cause of cancer-related death in women and it represents a major health burden worldwide. One of the promising breast cancer therapeutic avenues is through small molecule inhibitors (SMIs) which have undergone rapid progress with successful clinical trials. Recently, three emerging and vital groups of proteins are targeted by SMIs for breast cancer treatment, namely cyclin-dependent kinase 4 and 6 (CDK4/6), poly (adenosine diphosphate-ribose) polymerase (PARP) and phosphoinositide 3-kinase (PI3K). Several of these inhibitors have been approved for the treatment of breast cancer patients or progressed into late-stage clinical trials. Thus, modeling from these successful clinical trials, as well as their limitations, is pivotal for future development and trials of other inhibitors or therapeutic regimens targeting breast cancer patients. In this review, we discuss eight recently approved or novel SMIs against CDK4/6 (palbociclib, ribociclib and abemaciclib), PARP (olaparib, veliparib and talazoparib), and PI3K (buparlisib and alpelisib). The mechanisms of action, series of clinical trials and limitations are described for each inhibitor.

Keywords: breast cancer, CDK4/6 inhibitor, objective response rate, PARP inhibitor, PI3K inhibitor, progression-free survival

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Methods

For clinical trial studies, literature review was performed according to the electronic databases Google Scholar and MEDLINE with the following keywords: breast cancer, CDK4/6 inhibitor, PARP inhibitor, PI3K inhibitor, approved inhibitors, drugs, clinical trials, phase I, phase II, phase III, adverse events (AEs) and/or side effects. Abstracts and proceedings were included while reviews, editorials and case reports were excluded. Additional relevant studies were identified through a manual search of the bibliographic references of all retrieved articles. Ongoing and unpublished clinical trials were searched in ClinicalTrials.gov database (http://www.clinicaltrials.gov). No limit was applied for the following characteristics: types of treatment combinations or adjuvant therapies, their doses, HER2 status, breast cancer subtypes, and date of studies. Only English-language articles were reviewed. Significant findings were described based on the level of significance used in each individual study.

Results and discussion

CDK4/6 inhibitors

CDK4/6 play vital roles in the proliferation of mammalian cells. In particular, several tumorigenic events ultimately drive proliferation through CDK complexes, underscoring CDK4/6 as important therapeutic targets in cancer treatment. CDK4/6 interact with D-type cyclins to phosphorylate the retinoblastoma (Rb) tumor suppressor protein, thereby releasing E2F transcription factors that activate transcription of genes required for DNA replication and hence promoting the progression of cell cycle from the G1 phase to the S phase. Thus, inhibition of CDK4/6 blocks the phosphorylation of Rb where E2F remains bound as an inactive complex and is unable to activate the expression of genes that favor cell cycle progression.

CDK4/6 has also been shown to phosphorylate the forkhead box M1 (FOXM1) oncogenic transcription factor. In the study by Anders and colleagues, the authors performed a systematic screening for cyclin D1-CDK4 and cyclin D3-CDK6 substrates, and FOXM1 was positively regulated through direct phosphorylation by CDK4/6 (independently of Rb proteins), resulting in the stabilization of FOXM1. FOXM1 is critical for CDK4/6-mediated cell cycle entry and proposed to be a therapeutic target in breast cancer as it is overexpressed in the disease that contributes to therapy resistance. CDK4/6 inhibitors including palbociclib and ribociclib could decrease the levels of FOXM1 in breast cancer cells and other types of tumors, leading to induction of senescence and decreased cellular proliferation.

In addition, sufficient mitogenic signals drive the production of cyclin-D proteins that associate with and activate their catalytic partners to promote G1/S phase transition leading to cellular proliferation (Figure 2). CDK4/6 activity is also regulated by the INK4 family of proteins (p16INK4A, p15INK4B, p18INK4C, and p19INK4D) and by the Cip and Kip family (particularly p21CIP1 and p27KIP1), where the INK4 protein directly binds CDK and inhibits CDK4/6 activities.

Overexpression of CDK4/6 is considered a hallmark of several human malignancies with CDK4/6 and their subunit cyclin-D found to be associated with oncogenes in human cancers. Over two decades of research on CDK4/6 has demonstrated the significant efficacy of their inhibition in cancer therapy. Mouse models lacking D-type cyclins or CDK4/6 showed specific roles for these proteins in promoting cellular proliferation. Moreover, The Cancer Genome Atlas (TCGA) reported that amplification of cyclin-D1
(CCND1) occurred in 29% and 58% of luminal A and B breast cancers, respectively. Similarly, a higher proportion of luminal B subtype patients presented with a gain of CDK4 (25%) compared with luminal A patients (14%).

The pervasive nature of altered CDK4/6 and CCND1 expression has rendered them as attractive targets for anticancer therapies. Recently (2017), three SMIs targeting CDK4/6 have been approved including palbociclib (PD0332991), ribociclib (LEE011) and abemaciclib (LY2835219). These SMIs have demonstrated low half maximal inhibitory concentration (IC50) values (<40 nM) with antiproliferative activities against Rb-proficient human tumor in xenograft models. The effectiveness of each drug has been shown in a series of clinical trials with significant clinical benefits for breast cancer patients as discussed below and summarized in Table 1. For further details on the chemical structures, molecular mechanisms, IC50 against other CDK families of proteins, and preclinical studies of CDK4/6 inhibitors, readers are directed to the recent reviews by Lynce and colleagues and Xu and colleagues.

**Palbociclib.** Palbociclib (PD0332991) targets CDK4/6 and potently inhibits both CDK4/6 and cyclin-D1 activities. The inhibitor produces antiproliferative effects on Rb-positive cells in vitro and in several types of Rb-positive breast cancer cells. Palbociclib’s activity is associated with reduced Rb phosphorylation, leading to G1 arrest and reduced expression of the cell proliferation marker Ki-67.

**Clinical trials of palbociclib.** In phase I trial of palbociclib, 41 patients with Rb-positive advanced solid tumors including breast cancer were enrolled in the dose escalation study. Overall, 11 (27%) of the patients responded positively with a best response of stable disease (SD) for four cycles. Dose limiting toxicities (DLTs) were observed in
| SMI | Treatment | Patient population | Outcomes |
|-----|------------|--------------------|----------|
| Palbociclib | Single agent palbociclib | SMI Trial Phase | Overall median PFS: 3.7 months |
| | Palbociclib | ER-positive, HER2-negative breast cancer  (n = 37) | Clinical objective response before surgery: 97% |
| | Letrozole + palbociclib/letrozole alone | ER-positive, HER2-positive breast cancer (n = 33) | CR (40%) PR (45%)
| | Trastuzumab + pertuzumab + palbociclib + fulvestrant | ER-positive, HER2-negative invasive breast cancer patients (n = 66) | pCR: one patient EP score ( > 5) before treatment, (82%); after treatment (47%)
| | Palbociclib + letrozole | Palbociclib + letrozole/placebo + placebo | PFS: 24.8 months versus 14.6 months
| | | | PFS: 9.2 months versus 3.8 months
| | | | PCCA: C1D15 87% versus C1D12 6%; p < 0.001
| | | | PFS rate: 63% versus 37.1%; ORR: 51.7% versus 21.3%
| Ribociclib | Single agent ribociclib | HR-positive, HER2-negative advanced breast cancer  (n = 14) | Decrease in Ki-67 expression and phosphorylated Rb levels
| | Ribociclib | HR-positive, HER2-negative MBC | PFS: 23.8 months versus 16.9 months |
| | Ribociclib | HR-positive, HER2-negative MBC | ORR: 52.7% versus 41.9%
| Abemaciclib | Abemaciclib | Advanced breast cancer (n = 669) | PFS: 16.4 months versus 9.3 months ORR: 48.1% versus 21.3%
five patients that led to recommended maximum dose toxicity (MDT) of 125 mg daily and this MDT had been applied in an independent trial on Japanese patients that showed a positive tolerance. In a phase II clinical trial, 37 Rb-positive advanced breast cancer patients were administered with 125 mg palbociclib orally, and the patients achieved longer progression-free survival (PFS) when combined with endocrine therapy than endocrine therapy alone. The overall median PFS was 3.7 months and significantly longer for hormone receptor (HR)-positive patients compared to HR-negative patients and those with advanced disease treated with endocrine therapy. Furthermore, independent studies have shown improved efficacy of palbociclib when combined with other therapeutic agents including fulvestrant [a selective estrogen receptor (ER) degrader] or letrozole [a nonsteroidal aromatase inhibitor (NSAI)]. In a phase II randomized trial (PALOMA-1/TRIO-18) on advanced ER-positive, HER2-negative breast cancer patients receiving letrozole plus palbociclib (n = 84) and letrozole alone (n = 81), an improvement in the median PFS duration was observed with 29.6 and 27.9 months in the palbociclib plus letrozole and letrozole alone, respectively. This was followed by the PALOMA-2 phase III trial where palbociclib was combined with letrozole in patients with ER-positive, HER2-negative breast cancer. The combination of palbociclib plus letrozole resulted in pronounced improvement of PFS (24.8 months) compared with the placebo-letrozole group (14.5 months).

The pivotal phase III PALOMA-3 trial confirmed the efficacy of palbociclib combined with fulvestrant in patients with endocrine-resistant breast cancer regardless of the menopausal status. The trial demonstrated that PFS was substantially increased with a median PFS of 9.2 months (n = 347) and 3.8 months (n = 174) for patients receiving palbociclib-fulvestrant and placebo-fulvestrant, respectively. In both the PALOMA-1/TRIO-18 and PALOMA-3 trials, amplification of CCND1 and p16 loss did not predict clinical benefits of CDK4/6 inhibitors, and ER status was the best predictor to identify patients that responded positively to first-line CDK4/6 treatment. Although CCND1 amplification might not be predictive, high tumoral expression of its sister molecule cyclin E1 (CCNE1) showed worse PFS in metastatic breast cancer patients receiving palbociclib (PFS of 14.1 versus 7.6 months; p = 0.0024), suggesting that CCNE1 expression can be used to predict the efficacy of CDK4/6 inhibitors. In terms of Rb protein, independent phase II trials have demonstrated a lack of the association of clinical benefits with Rb status in breast cancer patients treated with palbociclib.

Limitations of palbociclib. The most common side effect of palbociclib observed was grade 3/4 neutropenia occurring in more than 60% of patients upon treatment with palbociclib alone or in combination with other drugs, while a minority (approximately 2%) of the patients developed febrile neutropenia. In the NA-PHER2 trial, grade 3 AEs were reported including neutropenia (29%), diarrhea (14%), increased aspartate aminotransferase (AST; 3%) and hypersensitivity reactions (3%). Furthermore, Chow and colleagues (phase II trial) reported that >50% of the patients developed febrile neutropenia with grade 3/4 neutropenia. DeMichele and colleagues proposed that palbociclib’s mechanism of myelosuppression may differ from that of traditional drugs where grade 3/4 neutropenia that occurred was largely uncomplicated, with one incident of fever and sepsis occurring in a neutropenic patient.

Other reported side effects were anemia, leukopenia, fatigue, nausea, and diarrhea. In addition, palbociclib has been associated with resistance that limits their cytostatic effects, causing cells to be arrested at G1 phase due to cyclin-D1 accumulation over prolonged exposure (72–96 h). This situation may be overcome by combining palbociclib with PI3K inhibitor (e.g. tamoxifen) which reduces cyclin-D1 and other G1-S cyclins, abolishing retinoblastoma protein (pRB) phosphorylation and subsequently inhibits the activation of S-phase transcriptional programs.

Ribociclib. Ribociclib (LEE011) is an orally bioavailable, selective SMI of CDK4/6 which obtained US FDA approval in March 2017. Ribociclib specifically inhibits CDK4/6 with IC50 at nanomolar range, and it is associated with the disruption of Rb protein phosphorylation, thereby preventing cell cycle progression and inducing G1 phase arrest. In preclinical settings, ribociclib exhibited significant tumor growth inhibition
HER2-negative advanced breast cancer patients showed significant clinical benefits in HR-positive, showing a clinically meaningful improvement in PFS earlier than planned as the primary endpoint of the MONALEESA-7 phase III trial, which was a crucial phase III trial that was terminated due to positive results. In the MONALEESA-2 phase III trial, the combination of ribociclib with letrozole in HR-positive, HER2-negative breast cancer patients demonstrated partial responses. The effectiveness of ribociclib and letrozole combination was tested in a phase II trial (MONALEESA-1) where HR-positive, HER2-negative breast cancer patients demonstrated a decrease of Ki-67 expression from baseline following treatment with either single agent letrozole or letrozole in combination with ribociclib. The combination led to decreased phosphorylated Rb levels and reduced CDK4, CDK6, CCND2, CCND3, and CCNE1 gene expression.

In the MONALEESA-2 phase III trial, the combination of ribociclib with letrozole or placebo plus letrozole was assessed as first-line treatment in postmenopausal women with HR-positive, HER2-negative recurrent or metastatic breast cancer who had not received previous systemic therapy for advanced disease. The trial showed a significant extension of PFS after 18 months in the ribociclib group compared with the placebo group, with a higher overall response rate of 52.7% in ribociclib than in placebo group. MONALEESA-2 was a crucial phase III trial that was terminated earlier than planned as the primary endpoint of clinically meaningful improvement in PFS was achieved.

The subsequent MONALEESA-7 phase III trial showed significant clinical benefits in HR-positive, HER2-negative advanced breast cancer patients. The trial assessed the combination of either ribociclib or placebo plus tamoxifen/NSAI and goserelin (a hormone-suppressing drug). The results showed that the ribociclib arm had improved PFS by almost two-fold [PFS: 13.0 months; ORR: 36%] in comparison with the placebo arm [PFS: 8.8 months; ORR: 36%].

Limitations of ribociclib. In a phase I study on ribociclib, common AEs included leukopenia, nausea and fatigue, however each AE occurred in less than 40% of the participants. Grade 3/4 neutropenia was reported in phase II (46%), phase III MONALEESA-2 (59.3%) and MONALEESA-7 (61%) trials, while no grade 3/4 AE was reported in a phase II trial. Greater rate of myelosuppression in the ribociclib group was also observed and AEs occurred due to DLTs as the dosage reductions permitted most patients to remain on treatment.

In the MONALEESA-2 trial, the ribociclib with letrozole combination produced side effects that infrequently occurred in treatment with other CDK4/6 inhibitors. Grade 1 and 2 infection of urinary and upper respiratory tracts occurred in 50.3% (n = 168/334) patients in the ribociclib group, while grade 3 urinary tract infection occurred in <1% of the patients. Other side effects observed were prolongation in QT interval, and elevation of liver enzymes such as alanine transferase (ALT) and AST levels in 9.3% and 5.7% of patients, respectively.

Abemaciclib. Abemaciclib (LY2835219) was approved by the US FDA in September 2017 as an inhibitor of CDK4/6 through oral administration. Abemaciclib displays a higher selectivity for CDK4 compared with CDK6, and it is multiple times more potent against CDK4/cyclin-D1 than CDK6/cyclin-D3 in enzymatic assays. Abemaciclib also exhibited higher inhibitory effects on CDK4 with IC50 of 2 nM compared with 9.9 mM for CDK6. The drug is a potent inhibitor of Rb phosphorylation as a single dose, and induces a complete cell cycle arrest and suppresses the expression of several Rb-E2F-regulated proteins. Abemaciclib is also a potential agent to treat breast cancer metastases to the brain which occurs in 30% of breast cancer patients due to the SMI’s higher ability to cross the blood–brain barrier.

Clinical trials of abemaciclib. A phase I study was conducted to evaluate the efficacy of abemaciclib as a single agent therapy in HR-positive breast cancer patients. In these patients, median duration of response was 13.4 months and median PFS was 8.8 months, along with decreased tumor size. Another phase I study in 55 patients with five different solid tumor types (breast cancer, non-small cell lung cancer, glioblastoma, melanoma and colorectal cancer) showed a tolerable safety profile. The phase II MONARCH 1 trial...
assessed the efficacy and related AEs of abemaciclib used as single-agent in heavily pretreated HR-positive, HER2-negative metastatic breast cancer patients \((n = 132)\). The trial concluded that the single-agent abemaciclib demonstrated promising clinical activity where ORR was 19.7\%, clinical benefit rate (CBR; i.e. complete response + partial response + stable disease \(\geq 6\) months) was 42.4\%, median PFS was 6.0 months, and median overall survival (OS) was 17.7 months.\(^40\)

In the phase III MONARCH 2 trial of advanced breast cancer patients \((n = 669)\) treated with abemaciclib combined with fulvestrant or fulvestrant alone, extended PFS was observed in the combination versus fulvestrant alone (median PFS 16.4 versus 9.3 months).\(^41\) In patients with measurable disease, abemaciclib plus fulvestrant achieved an ORR of 48.1\% compared with 21.3\% in the fulvestrant alone cohort. Lastly, the MONARCH 3 phase III trial was conducted in HR-positive, HER2-negative advanced breast cancer patients \((n = 493)\) to assess the efficacy of abemaciclib or placebo plus an NSAI (anastrozole or letrozole). The trial reported the median PFS in abemaciclib versus placebo arm (not reached versus 14.7 months). In addition, the reported ORR was 59\% in the abemaciclib arm and 44\% in the placebo group in patients with measurable disease.\(^37\)

**Limitations of abemaciclib.** The most common AEs rendered by abemaciclib was diarrhea that occurred in majority of the patients in the MONARCH 2 (86.4\%) and MONARCH 3 (81.3\%) trials, followed by neutropenia, leukenopenia, nausea and fatigue.\(^37,40,41\) The DLT of the drug was main DLT was neutropenia.\(^40\) This might be due to the mode of action of abemaciclib being more potent against CDK4 than CDK6.\(^40\)

Another potential limitation of abemaciclib is the possibility of acquired resistance. Long-term exposure of ER-positive cells (MCF-7) to abemaciclib led to the emergence of clones with amplified CDK6 expression and promoted abemaciclib resistance.\(^42\) However, its resistance in *in vivo* tumor models or in primary breast cancer patients remains to be defined.

**Adjuvant, neoadjuvant and HER2 settings of CDK4/6 inhibitors.** In terms of adjuvant strategies with each CDK4/6 inhibitor, the trials are in progress as follows: (1) Palbociclib: The PALLAS study (ClinicalTrials.gov identifier: NCT02513994) assessing the invasive disease free survival (iDFS) of HR-positive, HER2-negative early breast cancer patients \((n = 5600)\) randomized to receive adjuvant endocrine therapy with or without palbociclib with a primary completion date (PCD) in 2020; (2) Ribociclib: The EarLEE-1 (ClinicalTrials.gov identifier: NCT03078751) phase II trial assessing the safety and tolerability of ribociclib in combination with standard endocrine therapy in HR-positive, HER2-negative high risk early breast patients \((n = 2000); PCD: 2020; (3) Abemaciclib: The monarchE (ClinicalTrials.gov identifier: NCT03155997) phase III trial assessing the iDFS of high risk, node positive, HR-positive and HER2-negative early breast cancer \((n = 3580)\) randomized to receive standard adjuvant endocrine therapy with or without abemaciclib \((PCD: 2022)\).

In terms of neoadjuvant strategies, the phase II NA-PHER2 trial was conducted on 30 patients with ER-positive, HER2-positive breast cancer receiving a combination of trastuzumab, pertuzumab, palbociclib and fulvestrant.\(^28\) The combination demonstrated clinical benefits with clinical objective response achieved by 29 patients (97\%) before surgery, and pathological complete response (pCR) in breast and axillary nodes at surgery was achieved by 8 patients (27\%).\(^28\) Another neoadjuvant phase II trial on ER-positive, HER2-negative invasive breast cancer patients \((n = 20)\) reported that 17 (85\%) patients showed a clinical tumor response while 8 (40\%) and 9 (45\%) patients had complete response (CR) and partial response (PR), respectively.\(^29\) Analysis of the relative gene expression levels showed that all proliferative genes’ (*IL6ST, RBBP8* and *MKI67*) expression were decreased after the treatment.\(^29\)

The NeoPalAna (ClinicalTrials.gov identifier: NCT01723774) phase II neoadjuvant study was conducted on ER-positive, HER2-negative patients \((n = 50)\) receiving anastrozole for 4 weeks cycle (cycle 0) followed by palbociclib on cycle 1 day 1 (C1D1) for four 28-day cycles unless for patients with C1D15 Ki67 > 10\% due to inadequate response.\(^43\) In the study, the complete cell cycle arrest (CCCA; central Ki67 < 2.7\%) was significantly higher with the addition of palbociclib (C1D15 87\% versus C1D1 26\%; \(p < 0.001\)), indicating the efficacy of palbociclib in suppressing cell proliferation for patients resistant to anastrozole although prolonged administration was required.
Regarding HER2 settings, as HER2-positive breast cancer cells are responsive to CDK4/6 inhibitors in preclinical models, the NA-PHER2 phase II trial has also shown similar efficacy in combination with anti-HER2 antibodies and fulvestrant. A proportion of HER2-positive breast cancer patients display resistance towards anti-HER2 antibodies treatment, hence further clinical trials of CDK4/6 inhibitors in HER2-positive breast cancer patients are recommended as majority of the phase II/III trials of CDK4/6 inhibitors evaluated ER- or HR-positive but HER2-negative patients (Table 1).

**Future directions of CDK4/6 inhibitors.** In addition to clinical trials, further investigations on the mechanism of action (MoA) of each CDK4/6 inhibitor in breast cancer are required as recent studies have demonstrated a novel MoA of these inhibitors. For instance, it has been shown recently that palbociclib may have CDK4/6-independent antitumor activity. Palbociclib (but not ribociclib and abemaciclib) induced the apoptosis of human hepatocellular carcinoma (HCC) cells by inhibiting the PP5/AMPK (protein phosphatase 5/5’ AMP-activated protein kinase) axis independent of CDK4/6 activities. Similarly, in HCC and cholangiocarcinoma (CCA) cells, palbociclib (but not ribociclib) enhanced radiosensitivity of these cells through inhibition of ataxia telangiectasia-mutated (ATM) kinase and likely to be independent of CDK4/6 as suggested by the authors. In a proteome profiling study, palbociclib enhanced proteasomal activity of MCF7 breast cancer cells in a CDK4/6- and cell cycle-independent manner. These studies might explain the potency of palbociclib as an anticancer agent that targets both CDK4/6-dependent and independent pathways to attenuate cancer growth.

Although CDK4/6 inhibitors have rendered greater clinical benefits, patients tend to develop resistance to these drugs. One of the main resistance mechanisms is due to loss of Rb protein expression or its somatic mutations after exposure to CDK4/6 inhibitors (palbociclib or ribociclib) shown to occur in metastatic breast cancer patients, likely induced by selective pressure from CDK4/6 inhibitors treatment. Loss of Rb expression is more common in the TNBC subtype and hence CDK4/6 inhibitors are poor candidates for the treatment of TNBC patients. However, inhibition of both PI3K and CDK4/6 with SMIs has been reported in TNBC in preclinical settings in both *in vitro* and *in vivo* models with enhanced efficacy than either SMI alone. Other mechanisms of CDK4/6 inhibitors resistance include amplification of other CDKs (e.g. CDK6) and cyclins (e.g. CCND1), as well as induction of upstream mitogenic signals (e.g. FGFR), and combination therapies targeting these upregulated signaling pathways have been proposed to overcome SMI resistance.

**PARP inhibitors**

PARP is a vital nuclear enzyme that regulates cell survival through DNA repair, genomic stability maintenance and programmed cell death. Owing to its roles in regulating apoptosis and being frequently expressed in breast cancers, the protein represents an attractive therapeutic target in the malignancy. PARP inhibitors trap PARP proteins on damaged DNA, resulting in the cytotoxic PARP–DNA complexes. Trapping efficiency of distinct PARP inhibitors vary, and a previous study showed that trapped PARP–DNA complexes were detectable *in vitro* at concentrations consistent with clinical exposures. PARP1 cleavage by caspases is required during apoptosis that causes inactivation of its enzymatic activity and resulting in the release of two protein fragments (24 and 89 kDa). PARP is closely related with the breast cancer susceptibility gene 1 and 2 (BRCA1 and BRCA2) where these enzymes (PARP1, PARP2, and PARP3) act as mediators in repairing DNA upon single-strand breaks. BRCA1/2 are expressed in nonmalignant breast epithelial cells and other tissues where they assist in the repair of DNA damage or trigger cellular destruction if the damaged DNA cannot be repaired. If BRCA1/2 lose their functions due to mutations, the risk of breast, ovarian and other malignancies increases. Synthetic lethality (i.e. simultaneous disruption of two or more genes) caused by PARP and BRCA1/2 mutations consequently block DNA repair pathways, leading to selective death of BRCA-deficient cells. The synthetic lethality thus represents a vital mechanism of PARP inhibitors in breast cancer treatments (Figure 2).

The use of PARP inhibitors to target DNA repair deficiencies with the combination of systemic therapies has generated significant clinical interest. PARP inhibitors such as olaparib, veliparib and niraparib showed highly efficacious inhibitory activities with IC50 values reaching the low nanomolar range. Moreover, in mouse models,
Olaparib and veliparib were effective in delaying mammary tumor glands development by increasing genomic stability. Detailed information on the molecular mechanisms, preclinical studies, and applications in other cancer types of PARP inhibitors are described in recent reviews by Lord and Ashworth, and Robert and colleagues.

Olaparib. Olaparib (AZD2281) is one of the most extensively used PARP inhibitors of various members of the PARP family including PARP1, PARP2 and PARP3. Olaparib was associated with antitumor activity in BRCA-deficient breast cancer cell lines and was also proven effective in vivo in mouse models. Olaparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity that increases the formation of the PARP-DNA complex, resulting in disruption of cellular homeostasis and cell death. Olaparib has been approved by the US FDA to treat advanced ovarian cancer and recently (January 2018) approved to treat germline BRCA-mutated (gBRCAmut) metastatic breast cancer.

Clinical trials of olaparib. The efficacy of the combination of olaparib and carboplatin was shown in a phase I/II study in breast cancer patients with germline BRCA1/2 (gBRCA1/2) mutation carriers with a dose of 400 mg twice daily for one week on a 21-day cycle. Clinical benefits were reported in these patients with an ORR observed in 52.4% of the 42 patients and one patient (2.4%) achieved a complete response. The efficacy, safety and tolerability of olaparib in combination with paclitaxel were also investigated in another phase I trial with metastatic TNBC patients. All patients received 200 mg of olaparib (4 × 50 mg capsules twice daily) in combination with paclitaxel 90 mg/m² administered as an intravenous infusion. Promising clinical outcomes were reported with median PFS of 6.3 months and ORR of 33.3% for cohort 1 (n = 9), while cohort 2 (n = 10) achieved 5.2 months of median PFS and ORR of 40%. Cohort 1 and 2 patients differed as follows: In cohort 2, paclitaxel was omitted or delayed for patients who experienced first occurrence of grade ≥2 neutropenia, and they received rescue granulocyte colony-stimulating factor before paclitaxel treatment resumed if the absolute neutrophil count (ANC) returned to normal range, or both paclitaxel and olaparib dosing discontinued if the ANC remained low.

A phase II trial was conducted on olaparib alone as a single agent in patients with BRCA1/2 mutations and advanced breast cancer, divided into cohort 1 (n = 27) with a maximum tolerated dose (MTD; 400 mg twice daily) and cohort 2 (n = 27) with a lower dose (100 mg twice daily). The ORR in cohort 1 was 41% and was 22% in cohort 2, and the trial thus supported the potential of PARP inhibition in BRCA-deficient breast cancers. The OlympiAD randomized, open-label phase III clinical trial compared the efficacy of olaparib monotherapy with standard therapy (capecitabine, eribulin or vinorelbine in 21-day cycles). The trial was conducted in patients (n = 302) with gBRCAmut and HER2-negative metastatic breast cancer. Significant results were reported with a median PFS of 7.0 and 4.2 months (p < 0.001) and the response rate was 59.9% and 28.8% in the olaparib and the standard-therapy group, respectively.

Limitations of olaparib. Grade 3/4 AEs such as neutropenia (in approximately 40% of patients), anemia (15–22%) and thrombocytopenia (20%) was observed in two independent phase I trials of olaparib administration in breast cancer patients. In particular, greater incidence and severity of neutropenia led to decrease in paclitaxel dose intensity. Phase II trial side effects mainly occurred as grade 1/2 of fatigue and nausea where each occurred in 41% of patients while the phase III OlympiAD trial reported that the most common AEs occurred in 20% of patients who experienced anemia, nausea, fatigue, vomiting, neutropenia or leukopenia.

Veliparib. Veliparib (ABT-888) is an oral PARP inhibitor for both PARP1 and PARP2 with Kᵢ (inhibitory constant) of 5.2 and 2.9 nmol/L, respectively. It readily crosses the blood–brain barrier and inhibits DNA repair, potentiating the cytotoxicity of DNA-damaging agents. Veliparib does not show significant trapping activity and it is thought to have an enhanced therapeutic window in combination regimens where a trapping MoA is not required.

Clinical trials of veliparib. An initial phase I study on 35 patients with various advanced solid tumors including breast cancer was conducted to determine the MTD, DLT, pharmacokinetics and pharmacodynamics of veliparib in combination with the topoisomerase I inhibitor irinotecan. The trial showed that the MTD was 100 mg/m² irinotecan (days 1 and 8) combined with veliparib 40 mg twice daily (days 1–14) on a 21-day cycle, and veliparib reduced tumor PARP content at all dose levels in the presence of irinotecan.
Additionally, in comparison with irinotecan alone, the veliparib–irinotecan combination increased the expression of two nuclear biomarkers of DNA damage and repair (DDR) machinery that is, nuclear phosphorylated histone 2AX (γ-H2AX) and phosphorylated Nijmegen breakage syndrome 1 (pNBS1), suggesting that the modulation of DDR has taken place in the context of reduced PARP1/2 functions.74

In another phase I study, the efficacy of veliparib was observed in combination with carboplatin \((n = 27)\) or as a single agent \((n = 44)\) in patients with \(gBRCA1/2\) metastatic breast cancer.59 The trial showed that PFS was 8.7 months and OS was 18.8 months in the veliparib-carboplatin combination group compared with veliparib alone that resulted in PFS and OS of 14.4 months and 5.2 months, respectively. The authors concluded that veliparib as a single agent or in combination with carboplatin were safe at 150 mg dose and showed promising efficacy as PARP inhibitor.59

Another phase II study was conducted to examine the safety and efficacy of veliparib with carboplatin/paclitaxel (VCP), temozolomide (VT) and placebo plus carboplatin/paclitaxel (PCP) with a total of 290 patients with \(BRCA1/2\)-mutated breast cancer.75 VCP showed higher clinical benefits compared with PCP where the median PFS was 14.1 and 12.3 months, median OS of 28.3 and 25.9 months, and ORR 77.8% and 61.3%, respectively. The VT group exhibited worse outcomes with median PFS 7.4 months, median OS 19.1 months, and ORR 28.6%.75

The randomized phase II trial aimed to improve the efficacy of cytotoxic chemotherapy by assessing the efficacy of the veliparib–cyclophosphamide combination \((n = 21)\) versus cyclophosphamide alone \((n = 18)\) in patients with refractory TNBC.55 The authors concluded that the combination did not enhance the efficacy of the treatment with PR in only two patients in the combination arm and one patient in the single agent treatment, and no significant difference in the median PFS.55

A phase III trial (BrighTNess) was conducted in stage II–III TNBC, where the efficacy of paclitaxel plus carboplatin and veliparib \((n = 316)\), paclitaxel plus carboplatin \((n = 160)\), and paclitaxel alone \((n = 158)\) was evaluated.76 The combination conferring the most favorable outcomes was paclitaxel plus carboplatin and veliparib, showing a higher proportion of patients (168 patients; 53%) who achieved pCR than the paclitaxel-alone group (49 patients; 31%; \(p < 0.0001)\).76

**Limitations of veliparib.** The most common toxicities among the patients treated across all dose levels of the veliparib-irinotecan combination included diarrhea, as experienced by the majority of patients (63%), followed by nausea (60%), fatigue (60%), neutropenia and leukopenia (49%).74 Furthermore, the combination of veliparib and carboplatin showed an increase in toxicity compared with treatment with veliparib alone, in which grade 3/4 AEs reported were neutropenia (56%), anemia (29%), febrile neutropenia (15%), and thrombocytopenia (2%) throughout the treatment.76

**Talazoparib.** Talazoparib (BMN 673) is an investigational PARP inhibitor. Murai and colleagues showed that talazoparib inhibited PARP with an efficacy comparable to olaparib and rucaparib, but was approximately 100-fold more potent in trapping PARP-DNA complexes and with higher cytotoxicity (in combination with alkylating agents methyl methanesulfonate and temozolomide).73,77,78 Talazoparib exhibited selectivity towards tumor cells with \(BRCA1/2\) or \(PTEN\) gene defects in vitro with greater potency than other PARP1/2 inhibitors.79 In a xenograft model, talazoparib displayed remarkable antitumor activity against tumor cells harboring \(BRCA\) mutations or a \(PTEN\) deficiency.79

**Clinical trials of talazoparib.** A first-in-human phase I study had shown the efficacy of talazoparib as a single agent \((1.0\,\text{mg/day})\) in breast cancer patients \((n = 14)\) with a deleterious \(BRCA1/2\) mutation where 50% of the patients achieved ORR and it was well tolerated.77 The phase II study ABRAZO (two stage, two cohort) assessed talazoparib adopting the dosage established in the previous phase I study \((1.0\,\text{mg/day})\) in patients with locally advanced or metastatic breast cancer and a \(gBRCA1/2\) mutation followed by platinum-based therapy (Cohort 1, \(n = 49\)) or at least three platinum-free cytotoxic-based regimens (Cohort 2, \(n = 35\)).80 Talazoparib was more effective in Cohort 1 [ORR: 21%; duration of response (DOR): 5.8 months; CBR: 38%; PFS: 4.0 months] than in Cohort 2 (ORR: 37%; DOR: 3.8 months; CBR: 66%; PFS: 5.6 months).

In a subsequent phase III study \((n = 287)\), talazoparib \((1.0\,\text{mg/day})\) was compared with the physician’s choice of therapy (PCT) in patients with...
advanced breast cancer and a 9BRCA1/2-mutation.81 The PFS was significantly longer in talazoparib versus the PCT group (8.6 versus 5.6 months; \( p < 0.0001 \)) although no significant difference was observed in terms of OS (22.3 versus 19.5 months; \( p = 0.105 \)).

Limitations of talazoparib. The most common all grade AEs in the talazoparib group were anemia and nausea, that occurred in half of the patients in phase II and III, followed by fatigue in about 37–50% of the patients in all phases,77,80,81 Grade 3/4 hematologic AEs in all phases occurred in about 50–70% of the patients that comprised of anemia, neutropenia and thrombocytopenia. Although treatment with talazoparib produced greater clinical benefit compared with PCT, it rendered greater toxicities.81

Future directions of PARP inhibitors. As the MoA of PARP inhibitors involves DNA repair deficiencies, the majority of phase II/III clinical trials of PARP inhibitors (Table 2) have not directly compared the efficacy of these inhibitors with other DNA damaging agents such as platinum-based drugs. Interestingly, the ABRAZO phase II trial showed that patients receiving talazoparib previously treated with platinum-based therapy displayed worse ORR, CBR and PFS than the cohort of patients previously treated with nonplatinum regimens,80 suggesting that talazoparib showed reduced activity in platinum-resistant patients or that a proportion of the patients were resistant to both platinum-based agents and talazoparib.

Patients resistant to both platinum therapy and PARP inhibitors might harbor novel genetic lesions such as high expression levels of the really interesting new gene (RING)-deficient BRCA1 (Rdd-BRCA1 or RING-less BRCA1) proteins shown to render resistance to platinum-based agents and PARP inhibitors, and RING-less BRCA1 arises from \( BRCA1^{1855delAG} \) mutation.82,83 Identifying such a group of patients to receive alternative therapies targeting RING-less BRCA1 or its signaling pathway might avoid ineffective treatments and resistance phenotypes.

PI3K inhibitors

PI3K inhibitors represent one of the novel inhibitors for breast cancer treatment that have progressed into late-stage clinical trials. The PI3K enzyme phosphorylates the 3’OH group of phosphatidylinositol, causing the activation of serine-threonine kinases associated with cell growth and proliferation of tumor cells84 (Figure 2). An aberrantly activated PI3K pathway is a common occurrence in breast cancer.4

PI3K can be divided into three classes of enzyme isoforms: PI3Kα, PI3Kβ, and PI3Kγ.85 Somatic \( PIK3CA \) mutations are regularly found in solid tumors and TCGA recently profiled tumors from 825 breast cancer patients where \( PIK3CA \) gene was the most common somatic mutation in luminal breast cancers.15,86 In addition, PI3K pathway activation is a hallmark for HR-positive breast cancer cells resistant to endocrine therapy.87 For further information on the molecular mechanisms, preclinical studies, and combination of PI3K with mTOR inhibitors, recent reviews by Dey and colleagues88 and Bahrami and colleagues89 are recommended.

Buparlisib. Buparlisib (BKM120) is an orally available SMI that inhibits pan-class I PI3K [heterodimers consisting of catalytic p110 subunits (i.e. \( \alpha, \beta, \delta \) or \( \gamma \)) and regulatory subunit (i.e. p85α, p85β, p55γ, p101 or p84)] in the PI3K/AKT kinase signaling pathway in an ATP-competitive manner, thereby inhibiting the production of the secondary messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and activation of the PI3K signaling pathway.86,90,91 This disrupts tumor cell growth and survival in susceptible tumor cell populations.

Clinical trials of buparlisib. A phase I dose escalation study was conducted in patients with advanced solid tumors (n = 35) including breast cancer (n = 9; 26%) where buparlisib was well tolerated with an MTD of 100 mg/d.92 In a phase I trial of buparlisib in combination with capcitabine (n = 25), the combination was well tolerated by metastatic breast cancer patients, where several patients demonstrated prolonged responses.93

Another phase I study of buparlisib in combination with fulvestrant in ER-positive metastatic breast cancer patients (n = 31) was conducted after the preclinical investigation of buparlisib that yielded promising results in ER-positive breast cancer.86 The buparlisib-fulvestrant combination achieved a CBR of 58.6% and it was particularly significant in patients who received the combination as a first- or second-line endocrine treatment with an ORR of 80% and 20% for first- or second-line treatment, respectively. A
| SMI Trial | Phase | Patient population | Treatment | Outcomes |
|-----------|-------|--------------------|-----------|----------|
| Olaparib | II    | BRCA1/2 mutations and advanced breast cancer. | Single agent olaparib | ORR: 41% (Cohort 1); ORR: 22% (Cohort 2) |
| NCT02000622 | II | Refractory TNBC (n = 39) | Veliparib + cyclophosphamide/cyclophosphamide + veliparib | PR: two patients versus one patient; PFS: 2.1 months versus 1.9 months |
| NCT01945775 | III | Advanced breast cancer and a gBRCA1/2 mutation (n = 431) | Talazoparib/physician's choice | PFS: 8.6 months versus 7.2 months; ORR: 66% versus 52%; TCBR: 54% versus 31%; ORR: 21% versus 19%; DOR: 3.9 months versus 3.1 months |
| NCT02032277 | III | Stage II-III TNBC (n = 634) | Paclitaxel + carboplatin, veliparib + paclitaxel, and placebo + paclitaxel | pCR: 53% versus 31% |

**Table 2.** Summary of phase II/III trials of PARP inhibitors discussed in this review.
buparlisib–fulvestrant synergy was proven to be clinically active and safe for patients with metastatic ER-positive breast cancer with modest AEs related to dose toxicity.86

The phase Ib trial PIKHER2 aimed to determine the MTD for a recommended phase II trial (RP2D) using a combination of buparlisib and lapatinib.94 The study was conducted on 24 trastuzumab-resistant, advanced breast cancer patients with HER2-positivity, and the patients were administered daily with oral buparlisib and lapatinib. The authors reported an MTD of 80 mg/d of buparlisib and 1000 mg/d of lapatinib, and among the evaluable patients, one (4%) experienced CR while 18 (75%) patients had SD. Lastly, the CBR was 29% and disease control rate was 79%.94

The NeoPHOEBE phase II trial targeted HER2-positive breast cancer patients, the first group receiving a buparlisib-trastuzumab-paclitaxel combination \((n=25)\) and second group receiving a placebo-trastuzumab-paclitaxel combination \((n=25)\). A total of 21 patients (84%) of each group had wild type PIK3CA and 4 patients (16%) had mutant PIK3CA. Authors reported that the buparlisib combination demonstrated a pCR and ORR of 32% and 69%, respectively, while the placebo combination group exhibited a pCR and ORR of 40% and 33%, respectively. Another phase Ib/II trial was conducted on HER2-positive breast cancer patients \((n=50)\) assessing the safety profile and antitumor activity using a combination of buparlisib plus trastuzumab.86 An acceptable safety profile was reported with one patient (2%) that achieved CR and four patients (8%) had confirmed PR. However, the ORR did not meet the endpoint \((\text{ORR} \geq 25\%\)) and the ORR achieved was only 10% \((90\% \text{ CI}, 4.0–19.9)\).96

The BELLE-2 phase III clinical trial was conducted on HR-positive, HER2-negative breast cancer patients receiving buparlisib or placebo plus fulvestrant.97 In the trial, the median PFS was 6.8 months in the buparlisib group \((n=576)\) while it was only 4.5 months in the placebo group \((n=571)\). In PI3K pathway-activated patients \((n=372)\), the median PFS was 6.8 months and 4.0 months in the buparlisib and placebo group, respectively. In the subsequent BELLE-3 phase III clinical trial, patients with advanced breast cancer pretreated with endocrine therapy or mTOR inhibitors were evaluated, where the patients received a buparlisib-fulvestrant combination \((n=289)\) or placebo-fulvestrant \((n=143)\).98 The median PFS showed the buparlisib-fulvestrant group achieved a higher PFS of 3.9 months compared with the placebo-fulvestrant group’s 1.8 months. Both the BELLE-2 and BELLE-3 trials confirmed that buparlisib displayed significant efficacy when combined with fulvestrant.

**Limitations of buparlisib.** In a phase I trial of a buparlisib-capecitabine combination, the most common AEs (all grades) reported were nausea (56%), hand-foot syndrome (52%), mucositis (48%), diarrhea (40%), and rash (36%), while the highest grade AEs (grade 3) were diarrhea (12%) and elevated ALT and AST (12%).93 Psychiatric impairment occurred in 20% of patients who experienced grade 3/4 AEs.93 Another phase I trial with the combination of buparlisib-fulvestrant reported that the most common AEs were fatigue (38.7%), transaminases elevation (35.5%), rash (29%) and diarrhea (19.4%).86

In the NeoPHOEBE phase II trial, the incidence of serious AEs was greater in the buparlisib group compared with the placebo group at 36% versus 8%, respectively. There were nine patients (36%) in the buparlisib group that discontinued due to AEs.95 In the BELLE-2 and BELLE-3 phase III trials, the most common grade 3/4 AEs were increased ALT (22–25%), elevated AST (18%) and hyperglycemia (12–15%), and other AEs observed were rash, fatigue and hypertension (<10%).97,98

The phase II/III trial of BELLE-4 aimed to investigate the combination of buparlisib \((n=207)\) or placebo \((n=209)\) with paclitaxel in patients with HER2-negative locally advanced or metastatic breast cancer with no previous chemotherapy for advanced disease.99 The study concluded that the addition of buparlisib to paclitaxel produced no clinical benefit in which there was no improvement in median PFS (8.0 months in buparlisib group versus 9.2 months in placebo group), and this was partially attributable to a higher incidence of discontinuation of the treatment due to AEs. In this trial, serious AEs including pyrexia, pneumonitis and diarrhea were reported more frequently in the buparlisib group (30% of patients) than the placebo group (21%). The most frequent AEs in the buparlisib group were diarrhea (55%), alopecia (51%), rash (43%), nausea and hyperglycemia (41% each).
Alpelisib. Alpelisib (BYL719) is an orally bioavailable selective inhibitor of PI3K α signaling that specifically targets PI3Kα. Fritsch and colleagues reported that alpelisib possesses higher efficacy and wider safety profile than pan-class 1 PI3K inhibitor (buparlisib) in patients with mutated PIK3CA (IC₅₀ ~4nmol/l). The authors also demonstrated the compound activity and selectivity profile in mouse models with robust dose- and time-dependent inhibition of PI3K signaling, yielding promising therapeutic efficacy against PIK3CA-dependent tumors.

Clinical trials of alpelisib. A phase Ib trial was conducted in patients with ER-positive, HER2-negative metastatic breast cancer (n = 26) who did not receive endocrine therapy, and the aim was to define the safety and tolerability of letrozole in combination with alpelisib. The combination showed a positive tolerability with a maximum dose of 300mg/d where 35% of the patients remained on treatment for more than 6 months and 31% remained on treatment for more than 12 months.

A separate phase Ib/II trial was conducted on ER-positive, HER2-negative breast cancer patients to assess the efficacy in three arms that is, ribociclib-letrozole (n = 41), alpelisib-letrozole (n = 21) and ribociclib-alpelisib-letrozole (n = 36). The authors concluded that the ribociclib-alpelisib-letrozole combination showed an acceptable safety profile with a more consistent reduction in Ki-67 expression compared to the other two drug combinations, indicating the potential to target both CDK4/6 and PI3K signaling pathways as a more effective therapeutic strategy for breast cancer patients.

Limitations of alpelisib. The most common side effects of alpelisib were gastrointestinal disorders, hyperglycemia, fatigue and rash. About 30% of the patients did not benefit from alpelisib due to overexpression of FGFR1. The IC₅₀ of alpelisib was increased more than 10-fold in ER-positive or PIK3CA-mutant cells compared with cells without FGFR1 amplification. In addition, FGFR1 was amplified in approximately 10% of breast cancers associated with poor prognosis.

Alpelisib’s activity when used as monotherapy in patients with ER-positive breast cancer was limited by the induction of ER transcriptional activity prior to PI3K inhibition. This was demonstrated in xenografts with tumors of patients who underwent treatment with alpelisib where increased expression of genes containing ER binding sites and occupancy by ER at promoter regions were reported. The authors suggested that simultaneous ER suppression and PI3K inhibition is a potential therapeutic option for breast cancer patients.

Future directions of PI3K inhibitors. Brain metastasis (BM) is an end stage of breast cancer progression resistant to several treatment modalities and with low survival rates. Recently, Kodack and colleagues reported that BM mediated resistance to PI3K inhibition (buparlisib) through HER3 signaling pathway activation in a xenograft model with HER2-amplified or PIK3CA-mutant human breast cancer cells. The PI3K inhibitors resistance can be overcome by the synergistic treatment with pertuzumab, a therapeutic antibody that inhibits HER2-HER3 dimerization and hence HER3 activation. Proviral integration Moloney virus (PIM) kinase expression is also correlated with the clinical resistance of the PI3K inhibitor (alpelisib) in breast cancer, where the downstream PI3K effector activation is maintained by PIM kinase in an AKT-independent manner, and thus treatment with PIM/PI3K inhibitors is recommended.

Conclusion
A summary of the inhibitors discussed in this review including the patient population, treatment and outcomes are presented in Tables 1–3. Other emerging inhibitors not discussed in this review (due to space constraints) that target CDK4/6 (trilaciclib), PARP (rucaparib, niraparib and PI3K (taselisib, doctalisib) and undergoing or have completed phase II or III clinical trials in breast cancer patients either as single agent or in combination with other regimens are summarized in Table 4.

SMIs play critical roles in multiple aspects of biology associated with cellular proliferation and DNA transcription. A series of successful clinical trials have been reported, however the SMIs presented in this review contain limitations, including relatively short PFS (e.g. below 36 months) with common AEs such as neutropenia, fatigue, diarrhea and infections, while a handful of the inhibitors have shown acquired resistance that limit their efficacy. OS data of the SMIs in breast cancer treatments remain insufficient, and in some cases are not reported, and
Table 3. Summary of phase II/III trials of PI3K inhibitors discussed in this review.

| SMI   | Trial                  | Phase | Patient Population                  | Treatment                                                                 | Outcomes                                               |
|-------|------------------------|-------|-------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------|
| Buparlisib | NCT01816594 (NeoPHOEBE) | II    | HER2-positive breast cancer (n = 50) | Buparlisib + trastuzumab + paclitaxel/placebo + trastuzumab + paclitaxel | pCR: 32% versus 40%; ORR: 69% versus 33%               |
|       | NCT01132664            | II    | HER2-positive breast cancer (n = 50) | Buparlisib + trastuzumab                                                | CR: 2%; PR: 8%; SD: 40%; ORR: 10%; CBR: 14%           |
|       | NCT01572727 (BELLE-4)  | III   | HER2-negative locally advanced or MBC (n = 416) | Buparlisib + paclitaxel/placebo + paclitaxel | PFS: 8.0 months versus 9.2 months                      |
|       | NCT01610284 (BELLE-2)  | III   | HR-positive, HER2-negative breast cancer (n = 1147) | Buparlisib + placebo/placebo + fulvestrant | PFS: 4.0 months versus 6.8 months                     |
|       | NCT01633060 (BELLE-3)  | III   | Advanced breast cancer (n = 432)     | Buparlisib + placebo/placebo + fulvestrant  | PFS: 1.8 months versus 3.9 months                     |
| Alpelisib | NCT01872260          | II    | ER-positive, HER2-negative breast cancer (n = 253) | Ribociclib + letrozole/ alpelisib + letrozole/ ribociclib-alpelisib-letrozole | Ribociclib + alpelisib + letrozole showed acceptable safety profile, more consistent reduction in Ki-67 expression |

CBR, clinical benefit rate; CR, complete response; ER, estrogen receptor; HER2, human epidermal growth factor 2; MBC, metastatic breast cancer; NCT, ClinicalTrials.gov identifier; ORR, objective response rate; pCR, pathological complete response; PFS, progress-free survival; PI3K, phosphoinositide 3-kinase; PR, partial response; SD, stable disease.

Table 4. Summary of phase II/III trials of other breast cancer inhibitors targeting CDK4/6, PARP and PI3K.

| Pathway | SMI   | Trial                  | Phase | Patient population                  | Treatment                                                                 | Primary completion |
|---------|-------|------------------------|-------|-------------------------------------|---------------------------------------------------------------------------|---------------------|
| CDK4/6  | Trilaciclib | NCT02978716        | II    | Metastatic TNBC (n = 102)           | Trilaciclib + gemcitabine + carboplatin                                  | December 2018       |
| PARP    | Rucaparib | NCT02505048 (RUBY)  | II    | MBC (n = 41)                        | Single agent rucaparib                                                   | December 2017       |
|         |        | NCT00664781          | II    | Advanced or MBC or advanced ovarian cancer (n = 78) | Single agent rucaparib                                             | January 2015        |
|         |        | NCT01074970          | II    | TNBC and BRCA1/2 mutations (n = 135) | Rucaparib + cisplatin                                                   | June 2018           |
| Niraparib | NCT03368729 (BRAVO) | II    | Metastatic HER2-positive breast cancer (n = 40) | Niraparib + trastuzumab                                                 | June 2020           |
|         |        | NCT01905592          | III   | HER2-negative, germline BRCA mutation-positive breast cancer (n = 306) | Niraparib/physician’s choice                                           | May 2018            |

(Continued)
In conclusion, numerous trials have shown greater clinical benefits derived from multiple drug combinations than single agent therapies, and drug combinations could potentially hinder acquired resistance against a particular SMI. In view of the successful clinical trials targeting CDK4/6, PARP and PI3K pathways, we suggest that combination of SMIs targeting these three pathways represents a promising multidrug approach to treating breast cancer patients, and it warrants future expanded investigations.

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**Conflict of interest statement**

The authors declare that there is no conflict of interest.

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**Table 4. (Continued)**

| Pathway | SMI     | Trial            | Phase | Patient population                              | Treatment                                  | Primary completion |
|---------|---------|------------------|-------|------------------------------------------------|---------------------------------------------|-------------------|
| PI3K    | Taselisib | NCT02457910113   | II    | Androgen receptor-positive TNBC \( n = 73 \) | Taselisib + enzalutamide                    | December 2019     |
|         |         | NCT02273973 (LORELEI)114 | II    | ER-positive, HER2-negative early stage breast cancer \( n = 334 \) | Taselisib + letrozole /letrozole + placebo | September 2017    |
|         |         | NCT02340221 (SANDPIPER)115 | III   | Advanced or MBC \( n = 631 \) | Taselisib + fulvestrant /placebo + fulvestrant | July 2019         |
|         | Doctalisib | NCT01495247116  | II    | HER2-negative, inoperable locally advanced or MBC \( n = 18 \) | Doctalisib + paclitaxel                   | May 2014          |

CDK4/6, cyclin-dependent kinase 4 and 6; ER, estrogen receptor; HER2, human epidermal growth factor 2; MBC, metastatic breast cancer; TNBC, triple negative breast cancer; NCT, ClinicalTrials.gov identifier; PARP, poly [adenosine diphosphate-ribose] polymerase; PI3K, phosphoinositide 3-kinase; SMI, small molecule inhibitor.

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