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Review

CDK4/6 inhibitors in breast cancer: beyond hormone receptor-positive HER2-negative disease

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Abstract: The development of cyclin-dependent kinase (CDK) 4/6 inhibitors has been more prominent in hormone receptor (HR)-positive human epidermal growth factor receptor 2 (HER2)-negative breast cancers, with a significant improvement in progression-free survival (PFS) in first and later lines of metastatic breast cancer (MBC) therapy. Preclinical evidence suggests that there is activity of CDK4/6 inhibitors in nonluminal cell lines. Here, we present a review of the current preclinical and clinical data on the use of CDK inhibitors in HER2-positive and triple-negative breast cancer (TNBC).

Keywords: breast cancer, CDK4/6 inhibitor, HER2+ breast cancer, hormone receptor-negative breast cancer, triple-negative breast cancer

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Introduction

Limitless replicative potential is considered one of the hallmarks of cancer.[1] This has led to several therapeutic approaches that block dysregulated cell division. Cyclin-dependent kinase (CDK) 4 and 6 mediate transition from the G0/G1 phase to the S phase of the cell cycle.[2] They form a complex with cyclin D to promote the phosphorylation and therefore inactivation of the tumor suppression retinoblastoma (Rb) protein, allowing for cell division. Through inhibition of CDK4/6, Rb is dephosphorylated leading to cell cycle arrest.[2] Three of these CDK4/6 inhibitors (palbociclib, ribociclib and abemaciclib) have now been studied in various tumor types.[2] Since CDK4/6 activity is typically deregulated and overactive in breast cancer cells, these inhibitors offer an effective therapeutic approach against breast cancer.[3]

Most luminal breast cancers have upregulation of the Rb pathway, with a dependence on the CDK4/cyclin D1/Rb interaction.[3] Therefore, the development of CDK4/6 inhibitors has been particularly focused in hormone receptor (HR)-positive human epidermal growth factor receptor 2 (HER2)-negative breast cancers with a consistent and significant improvement in progression-free survival (PFS) in the first and later lines of metastatic breast cancer (MBC) therapy.[4–9]

Preclinical evidence suggests that there is activity of CDK4/6 inhibitors in nonluminal cell lines.[2,10–12] This, along with the ongoing need to improve the outcomes in MBC across different subtypes, has led to the evaluation of CDK4/6 inhibitors in HER2-positive and triple-negative breast cancer (TNBC) types. Here, we present a review of the current preclinical and clinical data on the use of CDK inhibitors in HER2-positive breast cancer and TNBC.

HER2-positive breast cancer

HER2 is overexpressed in approximately 20–25% of human breast cancers.[13] The development of the anti-HER2 therapies including trastuzumab, lapatinib, pertuzumab and trastuzumab emtansine (T-DM1) has undoubtedly improved outcomes in the MBC setting. The overall survival (OS) in the first-line setting of HER2-positive MBC impressively improved from 20.3 months with standard chemotherapy to now exceeding 50 months with the combination of chemotherapy with pertuzumab and trastuzumab.[14,15] In the

second-line setting, OS has also improved with integration of agents such as T-DM1 and the combination of lapatinib and trastuzumab.[16,17]

However, metastatic HER2-positive breast cancer remains an incurable disease, as patients eventually develop resistance to currently available treatment options and eventual disease progression and death.[18] Therapeutic strategies that prolong time to progression (TTP) by overcoming anti-HER2 resistance are critical to help continued improvement in HER2-positive breast cancer outcomes. Most resistance mechanisms identified for HER2-targeted therapies involve alterations to the receptor or upregulation or activation of various intracellular signaling cascades.[19–24] As CDK4/6 act downstream of many of these resistance mechanisms, it is desirable to evaluate the role of CDK4/6 inhibition to prevent or overcome the development of anti-HER2 resistance.[19–24] Preclinical data suggests that recurrent HER2-positive breast cancer cells are characterized by the loss of CDK inhibition and overexpression of cyclin D1, suggesting that cyclin D1-CDK4 activity has a critical role in developing resistance to HER2-pathway blockade.[24] Concomitant HER2 and CDK4/6 inhibition synergistically inhibits cell proliferation, controls tumor growth *in vivo*, and delays tumor recurrence in mice and *in vitro*.[24–26] These findings suggest the need to conduct clinical studies of CDK4/6 inhibitors in HER2-positive breast cancer.

Use of CDK inhibitors in HER2-positive MBC

Palbociclib. Palbociclib is the first studied oral CDK4/6 inhibitor, with the most mature data for safety and efficacy in the estrogen receptor (ER)-positive breast cancer subtype.[4,5] For HER2-positive breast cancer, palbociclib data in development is described below and in Table 1.

Reported results of palbociclib in HER2-positive MBC. A phase II study evaluating the use of palbociclib as a single agent for Rb protein-positive MBC has now been reported. Primary endpoints were disease response and tolerability; secondary endpoints included PFS and biomarker assessment to determine whether Rb localization, Ki-67 index, p16 loss, or cyclin D1 gene (CCND1) amplification were associated with response. Of the 37 patients enrolled, two patients (5%) had HR-positive HER2-positive disease and had not received prior HER2-directed therapy. Of these, one had a partial response and the other had a stable disease lasting 5 months on palbociclib. Biomarkers such as Rb nuclear expression, Ki-67 proliferation index, p16 loss, and cyclin D amplification were not associated with response.[27]

Studies underway with palbociclib in HER2-positive MBC in combination with approved anti-HER2 therapies. The phase II PATRICIA study is a prospective, open-label, multicenter trial testing the role of palbociclib and trastuzumab in HER2-positive MBC (ClinicalTrials.gov identifier: NCT02448420). Patients who also have HR-positive disease will also receive letrozole. This study aims to evaluate if the addition of palbociclib to standard therapy is well tolerated and can provide a benefit for patients with HER2-positive breast cancer previously treated for metastatic disease with at least two prior lines of therapy for advanced breast cancer.

Another ongoing phase II study is evaluating palbociclib in patients with HR-negative HER2-positive breast cancer with brain metastases (ClinicalTrials.gov identifier: NCT02774681). Patients may also receive trastuzumab as standard of care concurrently with palbociclib. In this single-arm study, the primary outcome is to determine the radiographic response rate in the central nervous system (CNS). Secondary endpoints are time to CNS progression, PFS, OS, objective response rate (ORR) and toxicity. Eligible patients should not have received more than two prior chemotherapy regimens for metastatic disease and the study excludes patients with uncontrolled neurologic symptoms from brain metastasis or patients with leptomeningeal disease.

Another study that is currently recruiting is a multicenter, single-arm, open-label phase I/II trial of anastrozole, palbociclib, trastuzumab, and pertuzumab as first-line therapy in metastatic HR-positive HER2-positive breast cancer patients (ClinicalTrials.gov identifier: NCT03304080). Primary outcomes include confirmation of dose-limiting toxicity, maximum tolerated dose (MTD) and clinical benefit rate (CBR), with secondary outcomes of PFS and adverse events (AEs).

A phase III study for HER2-positive patients, PATINA is currently enrolling (ClinicalTrials.gov identifier: NCT02947685). In this study, patients in the first-line setting will receive a minimum of four and maximum of eight cycles of induction treatment with anti-HER2 therapy (standard

Table 1. Trials with palbociclib in HER2-positive breast cancer.

Trial ID	Status	Phase	Arms	Population	Results	Primary outcome
Metastatic breast cancer						
(1) Studies with reported results						
DeMichele and colleagues[27]	Recruiting (arm 1: metastatic breast cancer) has stopped recruitment; arm 5: any tumor type if tissue tests positive for CCND1 amplification, CDK4/6 mutation, CCND2 amplification or any other functional alteration at the G1/S checkpoint is still recruiting	II	Single arm: palbociclib	Metastatic	Two patients (5%) HR-positive HER2-positive, no prior HER2-directed therapy. Of these, one had a partial response and the other had a stable disease lasting 5 months on palbociclib; Rb nuclear expression, Ki-67 proliferation index, p16 loss, and cyclin D amplification were not associated with response.	ORR; safety and tolerability
(2) Studies underway with approved HER2 therapies						
NCT02448420 (PATRICIA)	Recruiting	II	1. HR negative: palbociclib + trastuzumab 2. HR-positive: palbociclib + trastuzumab 3. HR-positive: palbociclib + trastuzumab + letrozole	Metastatic	Pending	PFS at 6 months
NCT02774681	Recruiting	II	Single arm: palbociclib	Metastatic with brain metastases, HR-negative	Pending	Radiographic response rate in CNS
NCT03304080	Recruiting	I/II	Single arm: anastrozole + palbociclib + trastuzumab + pertuzumab	Metastatic, HR-positive	Pending	Dose-limiting toxicity, MTD, CBR
NCT02947685 (PATINA)	Recruiting	III	Induction treatment with anti-HER2 therapy (standard chemotherapy + anti-HER2 therapy with trastuzumab and pertuzumab), followed by randomization: 1. Endocrine therapy + anti-HER2 therapy + palbociclib 2. Endocrine therapy + anti-HER2 therapy	Metastatic, HR-positive	Pending	PFS

(Continued)

Table 1. (Continued)

Trial ID	Status	Phase	Arms	Population	Results	Primary outcome
(3) Studies underway with other investigational drugs						
NCT03054363	Recruiting	Ib/roll-over phase II	Single arm: tucatinib + palbociclib + letrozole	Metastatic, HR-positive	Pending	AEs, PFS
NCT03065387	Recruiting	I	1. Neratinib + everolimus 2. Neratinib + palbociclib 3. Neratinib + trametinib	Metastatic, EGFR mutation/ amplification, HER2 mutation/ amplification or HER3/4 mutation	Pending	Dose-limiting toxicity, MTD
Early stage breast cancer						
(1) Studies underway with approved HER2 therapies						
Gianni and colleagues (NA-PHER2 trial)[23]	Unknown	II	Single arm: trastuzumab + pertuzumab + palbociclib + fulvestrant	Neoadjuvant, HR-positive	Mean Ki67 expression: 31.9 at baseline *versus* 4.3 at week 2 ($n = 25$; $p < 0.0001$); pCR 27%; common AEs: neutropenia (29%), diarrhea (14%)	Measure of Ki67 at baseline, at week 2, at surgery; changes in apoptosis biomarkers
NCT02907918 (PALTAN)	Recruiting	II	Single arm: palbociclib + letrozole + trastuzumab ± goserelin for a total of 16 weeks, consisting of four 28-day cycles; Definitive surgery within 3–5 weeks after the end of cycle 4; Letrozole + trastuzumab will continue until the day of surgery; Adjuvant therapy following definitive surgery at the discretion of the treating physician	Neoadjuvant, HR-positive	Pending	pCR

AEs, adverse effects; CBR, clinical benefit rate; CNS, central nervous system; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MTD, maximum tolerated dose; NCT, ClinicalTrials.gov identifier; ORR, objective response rate; pCR, pathologic complete response; PFS, progression-free survival; Rb, retinoblastoma.

Table 2. Trials with ribociclib and abemaciclib in HER2-positive breast cancer.

Trial ID	Status	Phase	Arms	Population	Results	Primary outcome
Ribociclib						
(1) Studies underway with approved HER2 therapies						
NCT02657343	Recruiting	I/II	1. Ribociclib + T-DM1 2. Ribociclib + trastuzumab 3. If HR-positive: ribociclib + trastuzumab + fulvestrant	Metastatic	Pending	Phase I: MTD phase II: dose; CBR
Abemaciclib						
(1) Studies with reported results						
Patnaik and colleagues[28]	Completed	I	Single arm: abemaciclib	Metastatic	PR 36%; SD 64%	Safety; tolerability
Fujiwara and colleagues[29]	Completed	I	Single arm: abemaciclib	Metastatic	One patient with HR-negative HER2-positive had a 30% decrease in tumor size	MTD; dose-limiting toxicity
(2) Studies underway with approved HER2 therapies						
NCT02675231 (monarcHER)	Active, not recruiting	II	1. Abemaciclib + trastuzumab + fulvestrant 2. Abemaciclib + trastuzumab 3. Trastuzumab + standard of care chemotherapy	Metastatic	Pending	PFS

CBR, clinical benefit rate; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MTD, maximum tolerated dose; NCT, ClinicalTrials.gov identifier; PFS, progression-free survival; PR, partial response; SD, stable disease; T-DM1, trastuzumab emtansine.

chemotherapy plus anti-HER2 therapy with trastuzumab and pertuzumab) and after that they will be randomized to endocrine therapy plus anti-HER2 therapy with or without palbociclib. The primary goal is to determine if the addition of palbociclib delays disease progression and secondary endpoints include OS, 3 and 5 years landmark survival, ORR, duration of response (DoR), CBR, safety and patient-reported outcomes.

Studies underway with palbociclib in HER2-positive MBC in combination with investigational anti-HER2 therapies. A single-center, nonrandomized, multi-arm phase I study of the pan-HER inhibitor neratinib given in combination with everolimus, palbociclib or trametinib in advanced cancer patients with epidermal growth factor receptor (EGFR) mutation/amplification, HER2 mutation/amplification or HER3/4 mutations is enrolling (ClinicalTrials.gov identifier: NCT03065387). The study will have three arms: neratinib plus everolimus; neratinib plus palbociclib, and neratinib plus trametinib. Patients are selected for each arm at the investigators' discretion based on tumor type and molecular aberrations present. The patients included need to have measurable advanced solid tumors with no available curative therapeutic options. The primary endpoint is safety with determination of the MTD and dose-limiting toxicities for each treatment arm. Secondary endpoints include pharmacokinetic and pharmacodynamic analysis along with preliminary antitumor efficacy.

A multicenter, single-arm, open-label phase Ib/rollover phase II study is recruiting metastatic HR and HER2-positive breast cancer patients for a triple-targeted drug combination (HER2-targeted small

molecule inhibitor tucatinib, CDK4/6 inhibitor palbociclib and aromatase inhibitor letrozole; ClinicalTrials.gov identifier: NCT03054363). The primary endpoint of this study is safety and PFS.

Ribociclib. Ribociclib was the second CDK4/6 inhibitor approved for HR-positive HER2-negative breast cancer and has shown similar efficacy outcomes in randomized trials for HR-positive HER2-negative breast cancer.[6] Nevertheless, data for HER2-positive tumors are not yet available. The current ribociclib data are described below and in Table 2.

Studies underway with ribociclib in HER2-positive MBC in combination with approved anti-HER2 therapies. An open-label, nonrandomized, parallel-assignment phase I/II study will test ribociclib in combination with trastuzumab or T-DM1 for advanced or metastatic HER2-positive breast cancer (ClinicalTrials.gov identifier: NCT02657343) with both HR-positive and HR-negative patients. There will be three separate cohorts ribociclib in combination with HER2-directed therapy (ribociclib plus T-DM1; ribociclib plus trastuzumab; and ribociclib plus trastuzumab plus fulvestrant for HR-positive HER2-positive breast cancer). The inclusion criteria vary per cohort, but overall allow for prior treatment with anti-HER2 agents and chemotherapy in the metastatic setting. Primary endpoints are MTD or confirmed dose for phase II evaluation, as well as CBR. Secondary outcomes are plasma concentrations of ribociclib, ORR, PFS, OS, frequency of biomarkers and frequency of AEs.

Abemaciclib. Abemaciclib is a CDK4/6 inhibitor with low nanomolar potency and greater selectivity for CDK4.[28] Phase I studies show a distinct side-effect profile with less myelosuppression/neutropenia and more gastrointestinal toxicity compared with palbociclib and ribociclib.[29–32] This safety profile allows for continuous dosing to achieve sustained target inhibition that has led to efficacy and its approval as monotherapy, combined therapy with fulvestrant and recently in combination with an aromatase inhibitor for HR-positive MBC patients.[7–9,33] There are also data showing that abemaciclib crosses the blood–brain barrier.[34] A preclinical study has shown that abemaciclib has a potent synergistic effect when used in combination with lapatinib in the inhibition of cell viability.[24] Ongoing and completed studies of abemaciclib for HER2-positive MBC are described below and in Table 2.

Reported results of abemaciclib in HER2-positive MBC. A phase I study of single-agent abemaciclib disease showed a clinical benefit for all 11 patients with HR-positive HER2-positive breast cancer [partial response 4 (36%); stable disease 7 (64%)].[32]

Another phase I study nonrandomized, single-arm, open-label, dose-escalation phase I study from Japan studied abemaciclib as a single agent for patients with advanced cancers. Among the included patients, there was only one patient with HR-negative HER2-positive breast cancer with evidence of response to this agent.[35]

Studies underway with abemaciclib in HER2-positive MBC in combination with approved anti-HER2 therapies. The monarcHER phase II randomized, multicenter, three-arm, open-label, trial is evaluating the effectiveness of abemaciclib plus trastuzumab with or without fulvestrant when compared with standard chemotherapy plus trastuzumab in women with HR-positive HER2-positive locally advanced or MBC (ClinicalTrials.gov identifier: NCT02675231). Patients are eligible after prior exposure to at least two HER2-directed therapies for advanced disease, including T-DM1, as well as prior exposure to taxane in any disease setting. The primary outcome is PFS and secondary outcomes include OS, ORR, DoR, CBR, quality of life, pain control and pharmacokinetics. The study is no longer recruiting patients and initial results are awaited.

Use of CDK inhibitors in the early stage setting of HER2-positive breast cancer. The NA-PHER2 multicohort, open-label, exploratory, phase II, neoadjuvant study evaluated triple combination therapy targeting the ER, HER2, and Rb pathways in HR-positive HER2-positive breast cancer to avoid the use of chemotherapy.[36] A total of 36 patients were studied with previously untreated, histologically confirmed, unilateral, invasive, HR-positive HER2-positive breast cancer. Patients were treated every 3 weeks with intravenous trastuzumab (8 mg/kg loading dose followed by 6 mg/kg) and intravenous pertuzumab (840 mg loading dose in the first cycle and then at 420 mg) for six cycles plus oral palbociclib (125 mg once a day for 21 days in a 4-week cycle) and intramuscular fulvestrant (500 mg) every 4 weeks for five cycles. The coprimary endpoints were change from baseline in Ki67 expression at 2 weeks of treatment and at surgery (16 weeks after treatment) and changes in

apoptosis from baseline to surgery. Secondary endpoints were clinical objective response and pathological complete response. In this study, the combination of palbociclib, fulvestrant, trastuzumab and pertuzumab had a significant effect on the expression of Ki67 at baseline *versus* week 2 [baseline mean Ki67 expression of 31.9 (SD 15.7) *versus* 4.3 (15.0) at week 2 ($n = 25$; $p < 0.0001$)]. A clinical objective response immediately before surgery was achieved by 97% of 30 patients and 27% of patients had a pathologic complete response (pCR). The most frequent grade 3 AEs were neutropenia (29%), diarrhea (14%), and stomatitis, increased alanine aminotransferase, and hypersensitivity reactions (3% of each event). No grade 4 or serious AEs were recorded in the study and there were no deaths.

PALTAN is an open-label, single-arm, phase II study evaluating the use of palbociclib in combination with neoadjuvant letrozole and weekly trastuzumab for a total of 16 weeks in newly diagnosed clinical stage II or III HR-positive HER2-positive breast cancer (ClinicalTrials.gov identifier: NCT02907918). This study is currently recruiting, and its primary endpoint is pCR rate, with safety and patient-reported outcomes as its secondary endpoints.

TNBC

TNBC is defined by the lack of expression of HR and HER2 and accounts for 10–20% of all breast cancers.[37] It is associated with poor outcomes when compared with other breast cancer subtypes.[38,39] While there has been improved understanding of TNBC tumor biology and classification of different subtypes, to date there is no targeted therapy approved based on these subtypes or identified biomarkers.[40,41] Therefore, promising treatment strategies for TNBC are needed and should be investigated.

Genomic, clinical and proteomic Rb pathway data in TNBC reveal 20% RB1 mutation or loss, 9% of cyclin E1 amplification, high expression of CDKN2A, low expression of RB1 and high proliferation rate.[3] Due to frequent Rb loss, TNBC patients are thus considered to be poor candidates for CDK inhibition.[3] However, TNBC was highly sensitive to CDK inhibition both *in vitro*, including one preclinical trial associating a CDK2/9 inhibitor with eribulin, and *in vivo*, suggesting that unknown factors in TNBC proliferation may still involve the CDK complex.[42,43]

Androgen receptor (AR)-positive TNBC may particularly be an interesting TNBC subtype for evaluation of CDK4/6 inhibitors as AR expression is significantly associated with Rb expression. Compared with Rb-negative TNBC, Rb-positive tumors were associated with older age, lower histologic grade and lack of BRCA1 mutation. These results suggest that clinically relevant factors may predict for Rb expression and potential targeted therapies in TNBC.[44]

Ongoing studies evaluating CDK4/6 inhibitors in TNBC are summarized below and in Table 3.

Use of CDK inhibitors in triple-negative MBC

Palbociclib. A phase I/II nonrandomized, open-label, single-arm trial is testing the safety and effectiveness of palbociclib with bicalutamide for the treatment of AR-positive TNBC (ClinicalTrials.gov identifier: NCT02605486). Treatment consists of bicalutamide orally once daily with a standard schedule of palbociclib. AR is considered positive if $\geqslant$1% of cell nuclei are immunoreactive. Primary outcomes include establishing the recommended phase II dose of combination treatment and 6 months PFS. Secondary outcomes are ORR, CBR, 1 year PFS, safety and tolerability. There is no limit to the number of prior regimens allowed.

The already mentioned phase II study evaluating the use of palbociclib as a single agent for Rb protein-positive MBC cited for HER2-positive patients has also included TNBC patients. Primary endpoints were disease response and tolerability; secondary endpoints included PFS and biomarker assessment to determine whether Rb localization, Ki-67 index, p16 loss, or cyclin D1 gene (CCND1) amplification were associated with response. Of the 37 patients enrolled, 4 patients (11%) had TNBC disease, all of whom rapidly progressed on treatment.[27]

Ribociclib. Ribociclib is also being tested in this setting in the BRE15-024 trial, an open-label, multi-institutional, single-arm phase I/II study of ribociclib in combination with bicalutamide in advanced AR-positive TNBC (ClinicalTrials.gov identifier: NCT03090165). Primary outcomes are MTD and CBR. Secondary outcomes are ORR, DoR, safety, pharmacokinetics, PFS and OS. Eligible patients need to have AR-positive TNBC with AR immunohistochemical staining >0% and one prior line of therapy is allowed.

Table 3. Trials with CDK inhibitors in TNBC.

Trial ID	Status	Phase	Arms	Population	Results	Primary outcome
(1) Studies underway evaluating palbociclib with other investigational drugs						
NCT02605486	Recruiting	I/II	Single arm: palbociclib + bicalutamide	Metastatic, androgen receptor-positive	Pending	Treatment dose, PFS
DeMichele and colleagues[27]	Recruiting	II	Single arm: palbociclib	Metastatic	All four patients with TNBC rapidly progressed on treatment	
(2) Studies underway evaluating ribociclib with other investigational drugs						
NCT03090165 (BRE15-024)	Recruiting	I/II	Single arm: ribociclib + bicalutamide	Metastatic, androgen receptor-positive	Pending	Phase I: MTD; phase II: CBR
(3) Studies underway evaluating abemaciclib						
NCT03130439	Recruiting	II	Single arm: abemaciclib	Metastatic, Rb protein-positive	Pending	ORR
(4) Others CDK inhibitors						
NCT02978716	Recruiting	II	1. Gemcitabine + carboplatin 2. Gemcitabine + carboplatin (days 1 and 8) + trilaciclib (days 1 and 8 of 21-day cycles); 3. Gemcitabine + carboplatin therapy (days 2 and 9) + trilaciclib (days 1, 2, 8, and 9 of 21-day cycles).	Metastatic	Pending	AEs
NCT01676753	Recruiting	Ib	Single arm: dinaciclib + pembrolizumab	Metastatic	Pending	MTD; dose-limiting toxicities
NCT01624441	Completed	I	Single arm: dinaciclib + epirubicin	Metastatic	Pending	MTD
NCT03519178	Recruiting	I/II	Single arm: PF-06873600	Metastatic	Pending	Dose escalation; safety and tolerability; ORR
NCT01333137	Terminated	I	1. Gemcitabine + carboplatin 2. Gemcitabine + carboplatin + P276-00	Metastatic	Pending	PFS

AEs, adverse effects; CBR, clinical benefit rate; CDK, cyclin-dependent kinase; MTD, maximum tolerated dose; NCT, ClinicalTrials.gov identifier; ORR, objective response rate; PFS, progression-free survival; Rb, retinoblastoma; TNBC, triple-negative breast cancer.

Abemaciclib. A single-arm open-label phase II trial is recruiting metastatic TNBC patients whose tumors express the Rb protein to receive single-agent abemaciclib (ClinicalTrials.gov identifier: NCT03130439). The invasive tumor must have >50% of tumor cells staining positive for Rb and patients may have received 1–3 prior chemotherapeutic regimens for MBC or may have developed recurrent/metastatic disease within 6 months of completing an anthracycline and taxane containing

(neo)adjuvant therapy regimen for TNBC. The primary endpoint is ORR and secondary endpoints are PFS, OS and CBR.

Other CDK inhibitors in metastatic TNBC

Studies underway with other CDK inhibitors in metastatic TNBC. Dinaciclib (SCH727965) is a novel and potent CDK inhibitor for CDK2, CDK5, CDK1 and CDK9, as well as thymidine (dThd) DNA incorporation.[45] Dinaciclib strongly suppresses phosphorylation of Rb.[46] Dinaciclib is active against various tumor cell lines including those beyond breast cancer.[46] Additionally, short exposures to dinaciclib were sufficient for long-lasting cellular effects.[45] Dinaciclib induced regression of established solid tumors in a range of mouse models following intermittent dosing schedule below the maximally tolerated level.[46]

An open-label phase Ib trial of weekly dinaciclib in combination with every 3-week pembrolizumab in patients with advanced TNBC is currently recruiting patients (ClinicalTrials.gov identifier: NCT01676753). Any number of prior therapies are allowed. Pembrolizumab will be administered every 3 weeks at a fixed dose of 200 mg IV. Dinaciclib will be administered on D1 and D8 of a 21-day cycle by 2-hour intravenous infusion. The starting dose level of dinaciclib in this study will be 12 mg/m^2. The primary objective is to define the MTD and recommended phase II dose of dinaciclib when given weekly in this combination and schedule. The primary endpoint is safety and tolerability. Secondary objectives include evaluation of the preliminary efficacy of this combination. Exploratory studies include characterizing and correlating PDL-1 and MYC overexpression with clinical response.

Another open-label, single-arm phase I clinical trial is now completed and analyzed the side effects and the best dose of dinaciclib when given together with epirubicin in patients with metastatic TNBC (ClinicalTrials.gov identifier: NCT01624441). Primary objectives included determination of MTD of dinaciclib given in combination with epirubicin and to determine the predictive value of myeloid cell leukemia sequence 1 protein (MCL-1), low molecular weight cyclin E (LMW-E), and tumor grade as predictors of biologic response. Secondary objectives included evaluation of the efficacy of combination therapy and to determine the effects of therapy on proliferation. Patients had no more than two prior chemotherapy regimens for MBC. Results are not yet available.

Another novel CDK inhibitor is PF-06873600, an orally bioavailable drug which is being studied in an open-label, multicenter, nonrandomized, multiple dose, safety, tolerability, pharmacokinetic, and pharmacodynamics and clinical activity phase I/II study. PF-06873600 will be administered in multiple tumors, including TNBC, as a single agent or in combination with endocrine therapy (ClinicalTrials.gov identifier: NCT03519178). TNBC patients are eligible if they have received up to two prior lines of chemotherapy. Primary endpoints are dose escalation, safety and tolerability and ORR. Secondary endpoints are the determination of drug concentration, DoR, PFS, TTP, OS and pharmacodynamic biomarkers.

A randomized, open-label phase I study analyzed P276-00, a small molecule, flavone-derived CDK4 D1, CDK1 B, and CDK9 T inhibitor, with potent cytotoxic effects against chemosensitive and chemoresistant cancer cell lines (ClinicalTrials.gov identifier: NCT01333137). It compared the efficacy of the standard chemotherapy regimen of gemcitabine and carboplatin when administered with or without P276-00 in patients with advanced TNBC. Patients received up to two prior chemotherapy regimens for advanced disease. The primary endpoint was PFS and secondary endpoints were OS, OS at 6 months, PFS at 6 months, ORR and DoR. Results are not yet reported.

Trilaciclib is an intravenous small molecule, competitive inhibitor of CDK4/6 with potential antineoplastic and chemoprotective activities. It may also protect all hematopoietic lineages, including red blood cells, platelets, neutrophils and lymphocytes, from the DNA-damaging effects of certain chemotherapeutics, preserving the function of the bone marrow and the immune system.[47] A phase II randomized, open-label trial is studying the role of trilaciclib in TNBC, randomizing patients between three groups: gemcitabine plus carboplatin therapy (days 1 and 8 of 21-day cycles); gemcitabine plus carboplatin therapy (days 1 and 8) plus trilaciclib on days 1 and 8 of 21-day cycles; and gemcitabine plus carboplatin therapy (days 2 and 9) plus trilaciclib on days 1, 2, 8, and 9 of 21-day cycles (ClinicalTrials.gov identifier: NCT02978716). The primary endpoint is the number of treatment-related AEs and secondary outcomes include tumor response, PFS, OS, hematologic parameters, pharmacokinetics, dose reductions and dose interruptions. Patients may have

received up to two regimens of chemotherapy in the metastatic setting.

Biomarkers to guide CDK inhibitors utilization

Classic chemotherapy drugs follow a general approach of untargeted and nonspecific cell damage. However, cancer treatment is currently being delivered in a personalized manner guided by molecular biomarkers. The increasing knowledge of genomic signatures is now able to guide the development and utilization of targeted therapy. Consequently, finding predictive biomarkers to targeted therapy, including CDK4/6 therapies, increases drug applicability and effectiveness while also spares patients from undesirable toxicity.

Multiple studies have tried to identify potential biomarkers for CDK inhibitors, such as HR status, CCND1 amplification or loss of p16, CDK4 or CDK6 levels, the cyclin E–CDK2 axis, Rb expression, Ki67 levels, ER gene (ESR1) mutations, PIK3CA mutations, and TP53 mutations.[48–51] However, to date, none of these biomarkers have a predicted response for HR-positive MBC patients. Recently, cyclin E levels was found to have a predictive value for CDK2-inhibition response in inflammatory breast cancer, as well as in preclinical data, where cells acquiring resistance to CDK4/6 inhibitors due to CCNE1 amplification could be resensitized by targeting CDK2.[51,52] In the PALOMA-3 trial, pretreatment cyclin E1 gene expression was predictive of response to CDK4/6 inhibition, with PFS in low cyclin E1 expression of 14.1 months *versus* 7.6 months for high cyclin E1 ($p = 0.00238$).[53]

Ongoing studies in the TNBC population and in the HER2-positive populations will possibly clarify the role of these biomarkers along with improved understanding of mechanism of action of CDK inhibitors when used in the HR-negative population.

Discussion

The use of CDK4/6 inhibitors has now been integrated in the care for HR-positive HER2-negative breast cancers.[4–9] Ongoing studies in HER2-positive and TNBC as outlined here will clarify whether CDK4/6 inhibitors have activity against these subtypes of breast cancer and ultimately improve the understanding of the mechanism of action of these drugs.

There are certainly key learning aspects from the development of CDK4/6 inhibitors in HR-positive breast cancer that we need to consider in application of design and conduct of the studies in HER2-positive and TNBC subtypes. Firstly, given the cost of these therapies, we need to ensure that cost-effectiveness analyses are integrated *a priori* in the study design. For instance, based on the PALOMA-2 trial, a Swiss cost-effectiveness study evaluated the burden of the addition of palbociclib to letrozole in MBC.[54] The results showed that a considerable price reduction for palbociclib would be needed to make the drug cost-effective, given the estimated additional annual cost of approximately US$22 million to the system. Of the trials reported in this review, there are three evaluating palbociclib in combination with the dual HER2-blockade: two in the metastatic setting (ClinicalTrials.gov identifier: NCT03304080; PATINA trial, ClinicalTrials.gov identifier: NCT02947685) and one in the neoadjuvant setting (NA-PHER2, ClinicalTrials.gov identifier: NCT02907918). These studies raise the question of whether these regimens (even if proven effective) will be economically viable and therefore applicable in clinical practice. Secondly, we must also be thoughtful in integrating translational research principles in the design of these studies. There is increased understanding that HER2-positive and certainly TNBC may represent a heterogeneous tumor population and as such early studies that evaluate tumor biopsies, circulating tumor DNA and other molecular techniques can identify specific subgroups and biomarkers to help enrich study population and guide trial design. For instance, palbociclib as monotherapy has not been shown to be associated with a response in TNBC tumors.[27] Although this study only recruited four patients with TNBC and therefore significant conclusions cannot be obtained, understanding TNBC tumor biology could lead to an improved approach in planning studies with CDK 4/6 inhibitors in this population. AR expression may also play a factor in predicting response of TNBC to CDK inhibition and consequently there is an ongoing trial assessing this patient population (ClinicalTrials.gov identifier: NCT02605486). Overall, the studies outlined in this review show that there is a rationale in developing trials with CDK inhibitors in nonluminal patients. HER-2 positive and TNBC have worse outcomes when compared with HR-positive breast cancer and more efficient therapies are needed. Overall, the available data suggest that HER2-positive breast cancer is the most promising among the nonluminal breast cancer subtypes to evaluate the role of CDK inhibition, although more

convincing data are still awaited before adoption in routine practice.

Another interesting perspective of the use of CDK inhibitors is the possibility to preclude chemotherapy in the neoadjuvant treatment of HER2-positive patients, as outlined in the NA-PHER2 study (ClinicalTrials.gov identifier: NCT02907918). For some vulnerable patient populations, such as elderly patients and those with low performance status, this strategy could be particularly compelling to avoid treatment-related toxicities. Nonetheless, more data are necessary to define the efficacy of such approach.

Furthermore, there are multiple other effective therapeutic classes and related trials underway for HER2-positive and TNBC subtypes. The integration of CDK4/6 inhibitors in this setting must be considered with a rational approach, especially understanding the mechanism of action of the different agents, potential for overlapping toxicity, evaluation of potential synergy and related strategies to either delay development of secondary resistance or evaluation of ideal delivery either in sequence or in combination approach. While there is certainly a need to improve patient survival outcomes, our goal should also include the achievement of meaningful benefits that improve or delay deterioration in quality of life.

In conclusion, integration of CDK inhibitors for HR-positive MBC has significantly improved the outcomes of our patients. We need to learn from these successes and improved understanding of tumor biology and mechanism of action of this class of drugs to optimally design studies for HER2-positive and TNBC subtypes. While the studies are just emerging for these subtypes, there is certainly strong hope that the efficacy potential of these therapies extends beyond HR-positive breast cancer and the onus is on us to design rational and scientifically sound studies that will assess meaningful benefit from CDK inhibition.

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Conflict of interest statement

SV declares membership on advisory boards for Amgen, AstraZeneca, Pfizer, Novartis, Roche, Spectrum, and Daiichi. AM and CA declare that there is no conflict of interest.

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