Review

Cyclin-Dependent Kinase 4/6 Inhibitors in Neoadjuvant Endocrine Therapy of Hormone Receptor-Positive Breast Cancer

Lorenzo Rossi,[1,2,3] Amelia McCartney,[1] Emanuela Risi,[1] Irene De Santo,[1] Ilenia Migliaccio,[1] Luca Malorni,[1] Laura Biganzoli,[1] Angelo Di Leo[1]

Abstract

The landscape of therapeutic options for the treatment of hormone receptor (HR)-positive (HR^+) $HER2^-$ breast cancer (BC) has been profoundly changed by the introduction of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors into the metastatic setting. Currently all CDK4/6 inhibitors are approved only in the metastatic setting by Food and Drug Administration (FDA) and European Medicine Agency (EMA), whereas their role in the neoadjuvant setting is still at an investigational stage. Exploitation of novel agents such as CDK4/6 inhibitors to improve the efficacy of neoadjuvant endocrine therapy (ET) or to overcome de novo resistance to ET is an area of research under active evaluation. We present a review of the currently available data and ongoing clinical trials that are evaluating the role of CDK4/6 inhibitors in neoadjuvant therapy of HR^+ $HER2^-$ early BC, and also illustrate translational aspects, such as the potential biomarkers of response to these new therapeutic agents.

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Background

The landscape of therapeutic options for the treatment of hormone receptor (HR)-positive (HR^+) $HER2^-$ breast cancer (BC) has been profoundly changed by the introduction of cyclin-dependent kinase (CDK) 4/6 (CDK4/6) inhibitors into the metastatic setting. These drugs target a molecular pathway, the cyclin D-CDK4/6-retinoblastoma tumor suppressor protein (Rb) axis, which is often altered in BC.[1,2] In particular, cyclin D-CDK4/6 complexes regulate the transition from the G1 to the S phase of the cell cycle, primarily by inactivating the Rb, therefore promoting cell proliferation.

Exposure to endocrine therapy (ET) causes sensitive BC tumor cells to show a reduction of cyclin D-CDK4/6 axis function.[3] However, as endocrine resistance develops, reactivation of the CDK4/6 pathway through different mechanisms is observed.[4] Additionally, molecular alterations in crucial components of the cyclin D-CDK4/6-Rb pathway (eg, gene amplification or overexpression of cyclin D1, or loss of function of negative pathway regulators such as p16 and p21) are commonly observed in luminal BC. Therefore, the cyclin D-CDK4/6-Rb pathway has for some time been considered a therapeutic target in luminal BC, prompting the development of CDK inhibitors. The first generation of such molecules proved unsuccessful because of toxicity issues and lack of selectivity.[5] Later-generation CDK inhibitors were developed with greater selectivity for the CDK4 and 6 isoforms, resulting in more effective and less toxic compounds.[6] To date, there are 3 CDK4/6 inhibitors available in clinical practice, palbociclib, ribociclib, and abemaciclib, which differ slightly in pharmacokinetic and toxicity profiles. All 3 agents have shown comparable efficacy in the first- and later-line metastatic setting, with an approximate doubling of progression-free survival compared with ET alone. After the results of the seminal PALOMA-1 (Palbociclib: Ongoing Trials in the Management of Breast Cancer),[7] PALOMA-2,[8] and PALOMA-3[9] trials, palbociclib was approved by the Food and Drug Administration (FDA) in the United States and by the European Medicines Agency (EMA) in Europe for the treatment of patients with HR^+ $HER2^-$ metastatic BC (mBC) in conjunction with aromatase inhibitors (AIs) or fulvestrant.[10,11] Ribociclib has been approved by the FDA and EMA for the same indications as palbociclib, on the basis of the results of the MONALEESA-2 (Mammary Oncology Assessment of LEE011's Efficacy and Safety),[12] MONALEESA-3,[13]

[1]"Sandro Pitigliani" Medical Oncology Department, Hospital of Prato, Prato, Italy
[2]Institute of Oncology of Southern Switzerland (IOSI), Bellinzona, Switzerland
[3]Breast Unit of Southern Switzerland (CSSI), Lugano, Switzerland

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Address for correspondence: Lorenzo Rossi, MD, Institute of Oncology of Southern Switzerland (IOSI), Bellinzona, Switzerland and Breast Unit of Southern Switzerland (CSSI), Lugano, Switzerland, Ospedale San Giovanni, 6500 Bellinzona, Switzerland
E-mail contact: lorenzo.rossi@eoc.ch

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and MONALEESA-7[14] trials. The phase III MONARCH trials[15,16] have tested the clinical efficacy and safety of abemaciclib, obtaining similar results in combination with AIs and fulvestrant. Currently all CDK4/6 inhibitors are approved only in the metastatic setting, whereas their role in the neoadjuvant setting is still at an investigational stage.

Neoadjuvant therapy (NAT) represents a standard treatment option for selected patients with locally advanced BC. The main goal of NAT has historically been to shrink inoperable lesions, in turn making them operable, and facilitating breast conservation without significant increase in local recurrence. Furthermore, NAT allows for early evaluation of response or resistance to therapy, in terms of reduction of the proliferation index (Ki-67 expression) or the achievement of complete pathological response (pCR). In the neoadjuvant setting systemic therapy is administered immediately, without having to wait for the time due to surgery and its after-effects (eg, healing, etc). In this sense the eradication of micro-metastatic foci through systemic therapy is "anticipated." Although chemotherapy (CT) is primarily used in NAT, use of ET in this setting has also been explored, particularly in postmenopausal women with luminal clinical stage II/III BC with strong estrogen receptor (ER) expression. However, in this context, ET rarely leads to pCR.[17] Exploitation of novel agents such as CDK4/6 inhibitors to improve the efficacy of neoadjuvant ET or to overcome de novo resistance to ET is an area of research under active evaluation. We present a review of the currently available data and ongoing clinical trials, on the role of CDK4/6 inhibitors in NAT of HR^+ $HER2^-$ early BC (eBC).

The Landscape of CDK4/6 Inhibitors in the Neoadjuvant BC Setting

The Role of ET Alone

The role of ET alone in BC neoadjuvant treatment had emerged in the early 1980s as an option for elderly women considered frail to be treated with CT or up-front surgery. Some of these trials compared the efficacy of AIs versus tamoxifen (eg, P024,[18] IMPACT [The Immediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen],[19] PROACT [Pre-operative Arimidex Compared To Tamoxifen],[20] STAGE [Neoadjuvant anastrozole versus tamoxifen in patients receiving goserelin for premenopausal breast cancer][21]), showing the superiority of AIs over tamoxifen. Most of these clinical trials suggested a duration of NAT from 3 to 4 months, but other studies (eg, Dixon and colleagues[22]) suggest that this duration could be insufficient to achieve maximum reduction of tumor size.

Direct comparisons of ET alone and CT in the neoadjuvant setting has only been addressed by 2 phase II trials. In the first trial, 239 postmenopausal women with stage IIA to IIIB HR^+ BC were randomized to receive AI for 3 months or CT containing doxorubicin with paclitaxel. The primary end point was pCR, but there were no statistically significant differences between the 2 arms of treatment in terms of pCR, clinical response rate, or time to response.[23] Similar results emerged from the GEICAM/2006-03 trial, which randomized 96 pre- and postmenopausal patients to receive exemestane with or without goserelin for 24 weeks or anthracycline- and taxane-containing CT. Clinical response rate (the primary end point of the trial) was not statistically significantly different between the 2 arms (23 patients [48%], in the exemestane group, 31 patients [66%], in the CT group; $P = .075$, with a trend for a worse outcome in the exemestane group for premenopausal patients).[24] The lack of robust data on the optimal typology and duration of the neoadjuvant ET, and on the comparison with the CT did not allow a wide use of the ET alone in the neoadjuvant setting; it was reserved for the particular cases in which CT was not feasible. In this context it is interesting to see if the additional use of CDK4/6 inhibition leads to significant results.

Results From Completed Trials on CDK4/6 Inhibitors With ET Alone or Compared With CT

Thus far, there are 5 clinical trials with available results that evaluated the efficacy of the combination CDK4/6 inhibitors with ET in the neoadjuvant setting. The phase II NeoPalAna (Neoadjuvant Palbociclib and Anastrozole for Clinical Stage 2 or 3 Estrogen Receptor Positive Breast Cancer) trial[25] was a single-arm study that enrolled 50 patients with stage II/III HR^+/$HER2^-$ eBC to initially receive anastrozole as monotherapy for 28 days, followed by the additional use of palbociclib over four 28-day cycles. In some patients, palbociclib use was then ceased with anastrozole monotherapy continued for 28 days before surgery. A subset of patients received a sixth cycle of dual palbociclib and anastrozole up to the point of surgery. The primary end point was comparative complete cell cycle arrest (CCCA), defined as Ki-67 <2.7% observed after 15 days of combination therapy (C1D15). The rate of CCCA at C1D15 was significantly higher compared with day 1 (87% vs. 26%; $P < .001$), regardless of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutational status and luminal subtype. Benefit from palbociclib was observed in tumors that were Grade 3 or negative for progesterone receptor. The most common adverse events were neutropenia (48% any Grade, 22% Grade 3, 4% Grade 4), leukopenia (22% any Grade, 22% Grade 2), and fatigue (14% any Grade, 14% Grade 2). The main limitation of this study was the lack of evaluation of pCR as a primary end point, and this limits the significance of the results.

The N007[26] was a phase II, single-arm, multicenter trial that enrolled 20 postmenopausal patients with HR^+ $HER2^-$ primary tumors (>2 cm in diameter) to receive neoadjuvant palbociclib and letrozole for 16 weeks. The co-primary end points were clinical response rate (with a radiological evaluation) and the changes in EndoPredict scores after combination treatment. The authors reported a clinical response of ≥50% in 17 patients including 8 complete responses. Furthermore, all lesions showed size reduction, all cancers were downgraded after treatment, and 1 patient achieved a pCR. Mean Ki-67 values were significantly reduced after treatment ($P = .044$). Additionally, mean EndoPredict scores were also significantly reduced compared with baseline measures ($P < .0001$). The safety profile was in line with previous data. Afebrile neutropenia occurred in all patients, predominantly after the first cycle, with more than half of those patients developing Grade 3/4 neutropenia requiring adjustment of dose or suspension of therapy.

The role of ribociclib as neoadjuvant treatment was explored in MONALEESA-1,[27] a 3-arm study that compared ribociclib

(at 2 doses: 400 or 600 mg/d) used with letrozole versus single-agent letrozole in 14 postmenopausal women with HR^+ $HER2^-$ Grade II/III disease who had at least 1 breast lesion of ≥1.0 cm. The primary end point was comparative Ki-67 levels between the 3 study arms. Indeed, Ki-67 levels were reduced in the 2 combination arms compared with letrozole alone (decrease of 69% in the letrozole monotherapy arm, 96% for letrozole with ribociclib 400 mg/d, and 92% for letrozole with ribociclib 600 mg/d). Combination therapy was well tolerated, with no reported Grade 3/4 adverse events. Unfortunately, the low number of patients does not allow us to be confident with these results.

The NeoMONARCH,[28] is a phase II study that enrolled 223 postmenopausal women with HR^+ $HER2^-$ primary breast tumor (≥1 cm) suitable for neoadjuvant endocrine monotherapy, randomized patients to receive either abemaciclib monotherapy, anastrozole monotherapy, or both in combination for 2 weeks, followed by 14 weeks of combination treatment. The primary end point was change in Ki-67 expression from baseline to the second week. Secondary end points were clinical, radiological, and pathological evaluation, safety profile, and pharmacokinetics. Ki-67 was reduced by 92.62% in the combination arm and 63.24% in the anastrozole monotherapy arm. Results on the abemaciclib monotherapy arm have not yet been published. The overall radiological response rate in all patients was 46.4%, with a 53.6% tumor size reduction in all patients. Only 3.7% of patients achieved pCR but the authors did not provide an explanation for such an unusually low rate of pCR. Grade 3 diarrhea occurred in 4% of patients, despite protocol-mandated prophylactic antidiarrheal therapy with loperamide. Other common adverse events were constipation (any Grade, 43.5%; Grade 3, 1.8%) and nausea (any Grade, 41.7%; Grade 3, 2.2%).

The results from the PALLET (Palbociclib in addition to Letrozole in neoadjuvant therapy in estrogen-receptor positive breast cancer; NCT02296801) study have been recently published.[29] Postmenopausal women (n = 307) with HR^+/$HER2^-$ tumors (>2 cm in size) were randomized to receive either single-agent letrozole for 14 weeks (arm A), single-agent letrozole for 2 weeks, followed by the additional use of palbociclib for 12 weeks (arm B), single-agent palbociclib for 2 weeks, followed by the additional use of letrozole (arm C), or letrozole with palbociclib for 14 weeks (arm D). During the interval between the 14th week of therapy and surgery, all patients continued letrozole treatment. The primary end points were clinical response and change in Ki-67 values observed in the 4 arms. The CCCA (defined as Ki-67 ≤2.7%), the pCR rate, safety profile, and the molecular/genetic profiles of tumor samples were among the secondary end points. Clinical response was not significantly different between the monotherapy arm versus combination therapy groups. Complete or partial responses were observed in 49.5% of the letrozole monotherapy arm, versus 54.3% in the combination groups ($P = .2$). However, the median log-fold change in Ki-67 expression was more evident in the combination groups versus letrozole alone (−4.1 vs. −2.2; $P < .001$). Also, CCCA was observed in 38 (58.5%) of 65 patients in the letrozole group compared with 113 (90.4%) of 125 in the combination groups ($P < .001$). This suggests that the additional use of palbociclib with letrozole enhanced the suppression of proliferation, but without an increase in clinical response. More patients had Grade 3/4 toxicity associated with combination therapy, compared with letrozole monotherapy (49.8% vs. 17%; $P < .001$). The main reported adverse event in the palbociclib with letrozole arms was asymptomatic neutropenia.

It remains to be seen whether the combination of CDK4/6 inhibitors used with ET might prove more efficacious than CT in the neoadjuvant setting. Currently, there are no definitive answers and many clinical trials are still ongoing. However, the results of the French NEOPAL (Efficacy of Letrozole + Palbociclib as Neoadjuvant Treatment of Stage II/IIIA PM50 ROR-defined Low or Intermediate Risk Luminal Breast Cancer, in Postmenopausal Women) trial have been recently published.[30] It was a phase II trial of comparison of standard neoadjuvant CT and ET used with palbociclib in 106 patients with HR^+ $HER2^-$ stage II to III BC. The primary end point was the frequency of residual cancer burden (RCB). The rate of patients with RCB 0/I (no or minimal residual disease) was higher in the CT arm compared with ET with palbociclib arm (15.5% vs. 7.7%), but response rate and breast conservation surgery rate were comparable. The median Ki-67 expression was significantly lower in the ET with palbociclib arm (3% vs. 8%; $P = .017$) and 2 serious adverse events occurred in the ET with palbociclib arm versus 17 in the CT arm, demonstrating a better safety profile of the ET with palbociclib group.

The main completed trials are summarized in Table 1.

Trials in Progress

Alongside these completed studies there are several ongoing trials of CDK4/6 inhibitors in the neoadjuvant treatment of HR^+ BC. PREDIX LUM A (NCT02592083) is a phase II trial in which pre- or postmenopausal patients with node-negative, luminal A (defined as ER^+ and progesterone receptor-positive ≥50% on immunohistochemistry, Ki-67 <20% in immunohistochemistry, and absence of HER2 amplification) tumors (<2 cm) initially receive neoadjuvant ET (tamoxifen or AIs with or without gonadotropin releasing hormone analogues) for 4 weeks. After this period, patients with a decrease in Ki-67 (≥20% of baseline) are randomized to continue single-agent ET, or to use palbociclib in addition at standard dosage, for a subsequent 12 weeks. Patients without a significant decrease in Ki-67 automatically receive the combination therapy with palbociclib with ET for 12 weeks. The primary outcome measures are clinical and radiological response. Secondary outcomes are disease-free survival, BC-specific survival, overall survival, safety and tolerability profiles, and quality of life measures. Other secondary end points include evaluation of changes in immunohistochemical characteristics observed in serial core biopsies. Preliminary results are expected in January 2019.

The FELINE trial (Letrozole plus Ribociclib or Placebo as Neoadjuvant Therapy in ER-positive, HER2-negative; NCT02712723) is a phase II trial aimed to determine if combined ribociclib with letrozole for 24 weeks as NAT increases the proportion of patients with a preoperative endocrine prognostic index (PEPI) score of 0 at surgery, compared with single-agent letrozole. PEPI is a tool for predicting the risk of recurrence in patients treated with neoadjuvant ET. The score is derived from the pathological T and N stage, Ki-67 level, and ER status of the surgical specimen. It has been previously validated in the IMPACT trial,[31] which showed no relapses occurred at 5 years among patients with a PEPI score of 0.

Table 1 Clinical Trials on CDK4/6 Inhibitors in the Neoadjuvant Setting With Available Results

Name of the Trial	Phase	Drugs	n	Patient Characteristics	Primary End Point(s)	Results
Additional Use of CDK4/6 Inhibitors With ET						
NeoPalAna	II	ANA (28 days) followed by ANA with P (4 cycles) vs. ANA with P (6 cycles)	50	Post-menopausal, ER^+ $HER2^-$, stage II/III	Complete cell cycle arrest (Ki-67 < 2.7%) after 15 days of therapy	87% vs. 26%, $P < .001$, in favor of the combination therapy arm
N007	II	L with P (16 weeks)	20	Postmenopausal, ER^+ $HER2^-$, T > 2 cm	Clinical response rate (radiological) and changes in EP score	Clinical response in 17 patients (8 clinical complete response)
MONALEESA-1	—	L with R 400 mg/d vs. L with R 600 mg/d vs. L	14	Postmenopausal, ER^+ $HER2^-$, stage II/III, T ≥ 1.0 cm	Ki-67 levels between the 3 study arms	Decrease of 69% in the L monotherapy arm, 96% for L with R 400 mg/d, 92% for L with R 600 mg/d
NeoMONARCH	II	ANA vs. ABE vs. ANA with ABE	223	Postmenopausal, ER^+ $HER2^-$, T ≥ 1.0 cm	Ki-67 expression from baseline to second week	Ki-67 reduction: 92.62% (in the combination arm), 63.24% (in the ANA monotherapy arm)
PALLET (NCT02296801)	II	L for 14 weeks (arm A); L for 2 weeks, followed by the additional use of P for 12 weeks (arm B); P for 2 weeks, followed by the additional use of L (arm C); or L with P for 14 weeks (arm D)	307	Postmenopausal, ER^+ $HER2^-$, T ≥ 2.0 cm	Clinical response rate and changes in Ki-67 expression	Clinical response rate 54.3% (combination arms) vs. 49.5% (L single-agent arm). Median log-fold change in Ki-67 expression −4.1 (combination arms) vs. −2.2 (L single-agent arm) $P < .001$
Comparison of CT With ET and CDK4/6 Inhibitors						
NEOPAL	II	L with P (19 weeks) or CT (FEC100 for 3 courses followed by docetaxel 100 mg/m^2 for 3 courses)	106	Postmenopausal, ER^+ $HER2^-$, luminal B or N^+ luminal A	Residual cancer burden rate	RCB 0/I/II/III in 3.8%/3.8%/52%/40.4% in the L with P arm, 5.9%/9.8%/37.3%/47.1% in the CT arm

Abbreviations: ABE = abemaciclib; ANA = anastrozole; CDK4/6 = cyclin-dependent kinase 4/6; CT = chemotherapy; EP score = EndoPredict score; ER = estrogen receptor; ET = endocrine therapy; FEC100 = fluorouracile-ciclofosfamide-epirubicin chemotherapy; L = letrozole; N^+ = nodal positive; P = palbociclib; R = ribociclib; RCB = residual cancer burden; T = tumor.

Similar to MONALEESA-1, the FELINE trial will evaluate 2 different schedules of ribociclib with letrozole: continuous ribociclib 400 mg/d, or ribociclib given at a standard "3 weeks on, 1 week off" schedule at 600 mg/d. The estimated study completion date is April 2023.

Three phase II clinical trials directly comparing standard CT and CDK4/6 inhibition used with ET are ongoing. First, the CORALLEEN trial (Neoadjuvant Multi-agent chemotherapy or Letrozole plus Ribociclib in Luminal B/HER2-negative Breast Cancer; NCT03248427) is a parallel, 2-arm trial in which postmenopausal patients with luminal-B BC (defined as $HR^+/HER2^-$ BC with Ki-67 levels ≥20%) are randomized to receive ribociclib (standard dosage) with letrozole versus standard CT (4 cycles of doxorubicin with cyclophosphamide [AC], followed by weekly paclitaxel [T], for 12 weeks). The primary end point is the evaluation of the rate of RCB. The study commenced accrual in July 2017 and 94 participants in total are expected. Ribociclib with letrozole is also being compared with standard CT (4 cycles of AC followed by weekly T for 12 weeks) in the NEOLBC trial (Tailoring NEOadjuvant Therapy in Hormone Receptor Positive, HER2 Negative, Luminal Breast Cancer; NCT03283384), in which patients with HR^+ $HER2^-$ stage II/III BC who do not obtain CCCA (the primary end point, defined by Ki-67 expression >1%) after 2 weeks of treatment with letrozole monotherapy (considered to be a surrogate marker of resistance to ET), will be randomized to receive additional ribociclib with letrozole, or to receive standard CT. The enrollment target is 100 patients and the estimated primary completion date is July 2020. Finally, PREDIX LUM-B (NCT02603679) is an ongoing trial in which the efficacy and toxicity of palbociclib with ET (tamoxifen or AIs with or without goserelin) versus CT (weekly paclitaxel 80 mg/m^2) for 12 weeks in patients with luminal B (defined as ER expression ≥20% and Ki-67 ≥20% and not HER2 amplified), stage II/III BC are being evaluated. The primary end point is the evaluation of radiological objective response rate using mammography and breast ultrasound or, alternatively, magnetic resonance imaging of the breast. After the initial 12-week period,

patients without radiological signs of progression are switched to the other arm for an additional 12 weeks, rather than proceeding to surgery. Radiological assessment is scheduled after every 6 weeks of treatment until the 24th week. In case of progression during treatment, surgery will be offered as primary treatment option.

In the specific subgroup of luminal BC with HER2 overexpression amplification ($HER2^+$), the TOUCH (To Reduce the Use of Chemotherapy in Elderly Patients With ER-positive and HER2-positive Breast Cancer) study (IBCSG 55-17), a randomized, multicenter phase II trial to evaluate the association between neoadjuvant palbociclib and HER2 blockade versus paclitaxel and $HER2^-$ blockade in elderly patients (older than 65 years old), is ongoing. The HER2 blockade is achieved with trastuzumab and pertuzumab with standard dosage and schedule. The primary end point is the evaluation of pCR rate (defined as absence of tumor cells in breast tissue and in lymph nodes at surgery time) in the 2 arms of treatment. Other end points are: pCR rate only in breast tissue, objective response before surgery (evaluated with clinical exams and/or imaging), tolerability, and conservative surgery rate. One hundred forty-four patients are expected and the expected duration of the study is 41 months after the randomization of the first patient (March 2022).

Triple blockade obtained with the administration of letrozole, palbociclib, and copanlisib (a phosphatidylinositol-3-kinase inhibitor) in HR^+ $HER2^-$ stage I to IV BC is being investigated in an ongoing phase I/II trial (NCT03128619) in which the safety profile and maximum tolerated dose of this triplet, in the (neo)adjuvant and metastatic settings are being evaluated.

Finally, the NCT02626507 trial is a dose-escalation phase Ib trial aimed to determine the toxicity, tolerability, and potential efficacy of gedatolisib (an inhibitor of phosphatidylinositol-3-kinase [PI3K] and mammalian target of rapamycin [mTOR] pathway) used in combination with palbociclib and fulvestrant as first treatment in 18 patients with newly diagnosed stage I to IV HR^+ $HER2^-$ BC. Preliminary results are expected in March 2019.

The main ongoing trials are summarized in Table 2.

Potential Biomarkers of Response

In the era of precision medicine, individualizing cancer treatment is a fundamental challenge in which identification of predictive biomarkers of response is paramount. It is beyond the scope of this review to describe all of the potential mechanisms of resistance to CDK4/6 inhibitors in BC, which has been achieved previously.[32,33] The current discussion is limited to potential biomarkers identified within CDK4/6 inhibitor NAT studies.

Preclinical studies have shown that CDK4/6 inhibitors exert their effects through the inhibition of cyclin D-CDK4/6-Rb-E2F pathway and cell cycle-related genes.[34-36] The POP (Randomized Phase II Study to Assess PD 0332991 in Breast Cancer) trial is a neoadjuvant, open-label phase II clinical trial that evaluated the antiproliferative

Table 2 Ongoing Clinical Trials of CDK4/6 Inhibitors in the Neoadjuvant Setting

Name of the Trial	Phase	Drugs	Estimated n	Patient Characteristics	Primary End Point
Comparison of Standard CT and CDK4/6 Inhibition With ET					
CORALLEEN (NCT03248427)	II	L with R vs. standard CT	94	Postmenopausal, ER^+ $HER2^-$	Rate of Residual Cancer Burden
NEOLBC (NCT03283384)	II	L with R vs. standard CT	100	Postmenopausal, ER^+ $HER2^-$	Complete cell cycle arrest (Ki-67 expression >1%)
PREDIX LUM-B (NCT02603679)	II	ET with P vs. paclitaxel	200	Pre- or postmenopausal, ER^+ $HER2^-$, nodal positive	Radiological objective response rate (mammography, breast ultrasound, or breast MRI)
TOUCH (IBCSG 55-17)	II	P with trastuzumab and pertuzumab vs. paclitaxel with trastuzumab and pertuzumab	144	Elderly (>65 years), ER^+ $HER2^+$	pCR rate (absence of tumoral cells in breast tissue and in lymph node at surgery time)
Additional Use of CDK4/6 Inhibitors With ET					
PREDIX LUM-A	II	TAM or AI with or without GnRH analogues for 4 weeks followed by ET single-agent or ET with P for 12 weeks	200	Pre- or postmenopausal, ER^+ $HER2^-$, luminal A, nodal negative, T $\geq$ 2 cm	Clinical and radiological response
FELINE	II	L vs. L with P for 24 weeks	120	Postmenopausal, ER^+ $HER2^-$, stage II/III	Changes in PEPI score (see text)
CDK4/6 Inhibitors With Other Targeted Therapies					
NCT02626507	Ib	Gedatolisib with P and fulvestrant	18	Pre- or postmenopausal, ER^+ $HER2^-$, stage I-IV	MTD
NCT03128619	I/II	Copanlisib with L and P	102	Pre- or postmenopausal, ER^+ $HER2^-$, stage I-IV	MTD; changes in Ki-67 expression after 2 weeks of treatment

Abbreviations: AI = aromatase inhibitor; CDK4/6 = cyclin-dependent kinase 4/6; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; GnRH = gonadotropin releasing hormone; L = letrozole; MRI = magnetic resonance imaging; MTD = maximum tolerated dose; P = palbociclib; pCR = pathologic complete response; PEPI = preoperative endocrine prognostic index; R = ribociclib; T = tumor; TAM = tamoxifen.

response to palbociclib via decrease in Ki-67 expression in patients who received palbociclib monotherapy for 15 days versus placebo. It showed that 50% of patients treated with palbociclib had a Ki-67 reduction, compared with 10% of patients treated with placebo.[37] Exploratory analyses of this trial specifically included a search for predictive biomarkers. As expected, palbociclib treatment led to a significantly higher decrease in Rb phosphorylation compared with placebo. This study also identified persistent elevated expression of CCND3, CCNE1, and CDKN2D at day 15 in patients resistant to palbociclib.[37] Similarly, the NeoPalAna trial showed that the PAM50 proliferation score[38] was significantly reduced after palbociclib treatment, and the N007 study showed gene expression of 3 proliferation-associated genes (*BIRC5*, *UBE2C*, and *DHCR7*) significantly decreased after 16 weeks of combination therapy.

Thymidine kinase-1 (TK1) plays a critical role in DNA synthesis and cell proliferation. Serum TK1 levels have been shown to act as a prognostic and biodynamic proliferation marker.[39,40] The effect exerted by palbociclib on cell proliferation has been shown in serum samples obtained from patients enrolled in the NeoPalAna trial, which analyzed serum TK1 activity on palbociclib.[41] A striking reduction in TK1 activity was observed after 2 weeks of palbociclib treatment in the NeoPalAna trial with a high concordance between changes in serum TK1 and tumor Ki-67.[41] After palbociclib withdrawal there was a significant increase of TK1 level from the 15th day of therapy to surgery because of recovery of CDK4/6 inhibition. In parallel, a rebound of Ki-67 expression was observed. However, when palbociclib was given longer (up to 5 cycles) the TK1 activity level remained suppressed until surgery.

Although it is intuitive that cell cycle-associated genes might serve as pharmacodynamic markers of CDK4/6 inhibitor treatment, they might also be used as predictive marker of response to these agents. An interesting exploratory analysis conducted within the NeoPalAna trial was the evaluation of the levels of mRNA gene expression of G1−S cell-cycle regulators, to test the hypothesis that resistance to palbociclib could be a result of deregulation of such genes. Indeed, a significantly elevated expression of CCND3, CCNE1 and CDKN2D (all regulated by E2F1, the major downstream effector of the CDK4/6 axis) was shown after 2 weeks of palbociclib treatment in the palbociclib-resistant group (defined by authors as non-CCCA after 15 days of palbociclib exposure), suggesting that persistent E2F1 activity could be a biomarker of poor response.[25] Additionally, in the POP trial, in patients treated with palbociclib, CCNE2 expression was significantly more decreased in responders to antiproliferative therapy versus nonresponders ($P < .006$).[37] In the NeoMONARCH trial, tumors resistant (defined as absence of radiological response after 15 days of exposure to the combination of anastrozole with abemaciclib) showed persistently elevated levels of cell cycle-associated genes compared with sensitive tumors.[28]

Conflicting results have been reported regarding the role of cyclin D1 overexpression/amplification and p16 expression. In the metastatic setting, the PALOMA-1 trial did not show differences in benefit from CDK4/6 inhibition on the basis of CCND1 amplification, as well as on p16 expression.[7] Consistent with these negative findings, the presence of CCND1 amplification and p16 expression were not predictive of antiproliferative response to palbociclib versus control in the POP trial.[37] Palbociclib response was independent of expression of *CCND1* and *CDKN2A* (p16) genes in the NeoPalAna trial,[25] whereas in the POP trial, the baseline cyclin D1 H-score predicted Ki-67 change (interaction test $P = .046$).[37]

The retinoblastoma gene product (Rb) is a regulator of cell proliferation, and a fundamental target of CDK4/6 inhibitors.[42] Loss of RB1 function has been associated with resistance to CDK4/6 inhibitors in vitro, but clinical data remain controversial.[32] Rb and phosphorylated Rb expression were not significantly associated with palbociclib-mediated antiproliferative response in the POP trial,[37] and 2 BCs carrying a mutation in RB1 were still sensitive to palbociclib in the NeoPalAna trial.[25] Conversely, RB1 gene expression levels were associated with sensitivity to abemaciclib in the NeoMONARCH trial.[28] Additional factors besides Rb might be implicated in resistance to CDK4/6 inhibitors. A gene expression signature of RB1 loss of function (RBsig), which is enriched for cell cycle-associated genes, has been shown to be a predictive biomarker of resistance to palbociclib in BC cell lines.[43] Interestingly, in the NeoMONARCH trial, RBsig expression levels were associated with sensitivity to abemaciclib, suggesting that it might be used to predict response to abemaciclib and endocrine treatment in HR^+ $HER2^-$ eBC.[28]

Finally, some results concerning markers of response to abemaciclib in the NeoMONARCH trial were presented in San Antonio Breast Cancer Symposium 2018. These findings showed that in anastrozole-resistant tumors at 2 weeks of treatment, the additional use of abemaciclib induced a cell cycle inhibition in most of the tumors. The authors concluded suggesting that the early changes in CCAG gene expression and baseline gene expression pathways might be used to predict response to abemaciclib and ET in this particular population.[44]

A recent analysis of the expression of the ratio of CCNE1/RB1 showed correlation with palbociclib half maximal inhibitory concentration in different data sets of BC and non-BC cell lines. The CCNE1/RB1 ratio could also be used to discriminate palbociclib sensitivity versus resistance among patients enrolled in the NeoPalAna trial, performing better than CCNE1 or RB1 alone.[34]

Conclusion

The CDK4/6 inhibitors are among the most innovative drugs introduced to the management of BC in recent years. Although in clinical practice their use is currently confined to the treatment of luminal mBC, interesting data are emerging in the neoadjuvant setting wherein they could potentially represent a compelling option in place of CT, with good efficacy and better tolerance, although head-to-head clinical trials are still to be conceived. Unfortunately, no reliable biomarker is yet available to select patients with HR^+ $HER2^-$ BC for CDK4/6 inhibitor treatment.

Disclosure

Angelo Di Leo reports consultant for Pfizer, Novartis, and Lilly; Luca Malorni, consultant for Pfizer, Novartis, and AstraZeneca. The remaining authors have stated that they have no conflicts of interest.

References

1. Murphy CG, Dickler MN. The role of CDK4/6 inhibition in breast cancer. *Oncologist* 2015; 20:483-90.
2. Arnold A, Papanikolau A. Cyclin D1 in breast cancer pathogenesis. *J Clin Oncol* 2005; 23:4215-24.

3. Miller TW, Balko JM, Fox EM, et al. ERalpha-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. *Cancer Discov* 2011; 1:338-51.
4. Osborne CK, Shiff R. Mechanisms of endocrine resistance in breast cancer. *Annu Rev Med* 2011; 62:233-47.
5. Asghar U, Witkiewicz AK, Turner NC, et al. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat Rev Drug Discov* 2015; 14:130-46.
6. Musgrove EA, Caldon CE, Barraclough J, et al. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer* 2011; 11:558-72.
7. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole vs. letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol* 2015; 16:25-35.
8. Finn RS, Martin M, Rugo HS, et al. Palbociclib and letrozole in advanced breast cancer. *N Engl J Med* 2016; 375:1925-36.
9. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib vs. fulvestrant plus placebo for treatment of hormone receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, doubleblind, phase 3 randomised controlled trial. *Lancet Oncol* 2016; 17:425-39.
10. Beaver JA, Amiri-Kordestani L, Charlab R, et al. FDA approval: palbociclib for the treatment of postmenopausal patients with estrogen receptor-positive, HER2-negative metastatic breast cancer. *Clin Cancer Res* 2015; 21:4760-6.
11. Walker AJ, Wedam S, Amiri-Kordestani L, et al. FDA approval of palbociclib in combination with fulvestrant for the treatment of hormone receptor-positive, HER2-negative metastatic breast cancer. *Clin Cancer Res* 2016; 22:4968-72.
12. O'Shaughnessy J, Petrakova K, Sonke GS, et al. Ribociclib plus letrozole vs. letrozole alone in patients with de novo HR^+, HER2− advanced breast cancer in the randomized MONALEESA-2 trial. *Breast Cancer Res Treat* 2018; 168:127-34.
13. Slamon DJ, Neven P, Chia S, et al. Phase III randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: MONALEESA-3. *J Clin Oncol* 2018; 36:2465-72.
14. Tripathy D, Im SA, Colleoni M, et al. Ribociclib plus endocrine therapy for premenopausal women with hormone receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. *Lancet Oncol* 2018; 19:904-15.
15. Sledge GW, Toi M, Neven P, et al. MONARCH 2: abemaciclib in combination with fulvestrant in women with HR^+/$HER2^-$ advanced breast cancer who had progressed while receiving endocrine therapy. *J Clin Oncol* 2017; 35:2875-84.
16. Goetz MP, Toi M, Campone M, et al. MONARCH 3: abemaciclib as initial therapy for advanced breast cancer. *J Clin Oncol* 2017; 35:3638-46.
17. Haddad TC, Goetz MP. Landscape of neoadjuvant therapy for breast cancer. *Ann Surg Oncol* 2015; 22:1408-15.
18. Ellis MJ, Ma C. Letrozole in the neoadjuvant setting: the P024 trial. *Breast Cancer Res Treat* 2007; 105(suppl 1):33-43.
19. Smith IE, Dowsett M, Ebbs SR, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J Clin Oncol* 2005; 23:5108-16.
20. Cataliotti L, Buzdar AU, Noguchi S, et al. Comparison of anastrozole vs. tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. *Cancer* 2006; 106:2095-103.
21. Masuda N, Sagara Y, Kinoshita T, et al. Neoadjuvant anastrozole vs. tamoxifen in patients receiving goserelin for premenopausal breast cancer (STAGE): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2012; 13:345-52.
22. Dixon JM, Renshaw L, Macaskill EJ, et al. Increase in response rate by prolonged treatment with neoadjuvant letrozole. *Breast Cancer Res Treat* 2009; 113:145-51.
23. Semiglazov VF, Semigalazov VV, Dashyan GA, et al. Phase 2 randomized trial of primary endocrine therapy vs. chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. *Cancer* 2007; 110:244-54.
24. Alba E, Calvo L, Albanell J, et al. Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. *Ann Oncol* 2012; 23:3069-74.
25. Ma CX, Gao F, Luo J, et al. NeoPalAna: neoadjuvant palbociclib, a cyclin-dependent kinase 4/6 inhibitor, and anastrozole for clinical stage 2 or 3 estrogen receptor-positive breast cancer. *Clin Cancer Res* 2017; 23:4055-65.
26. Chow LWC, Morita S, Chow CYC, et al. Neoadjuvant palbociclib on ER^+ breast cancer (N007) clinical response and EndoPredict's value. *Endocr Relat Cancer* 2018; 25:123-30.
27. Curigliano G, Gomez Pardo P, Meric-Bernstam F, et al. Ribociclib plus letrozole in early breast cancer: a presurgical window-of-opportunity study. *Breast* 2016; 25: 191-8.
28. Martin M, Hurvitz SA, Chan D, et al. Final results of NeoMONARCH: a phase 2 neoadjuvant study of abemaciclib in postmenopausal women with hormone receptor positive (HR^+), HER2 negative breast cancer (BC). *Cancer Res* 2018; 78 (abstract PD5-01).
29. Johnston S, Puhalla S, Wheatley D, et al. Randomized phase II study evaluating palbociclib in addition to letrozole as neoadjuvant therapy in estrogen receptor-positive early breast cancer: PALLET trial. *J Clin Oncol* 2019; 37: 178-89.
30. Cottu P, D'Hondt V, Dureau S, et al. Letrozole and palbociclib vs. 3rd generation chemotherapy as neoadjuvant treatment of luminal breast cancer. Results of the UNICANCER-eoPAL study. *Ann Oncol* 2017; 28(suppl 5): v605-49 (abstract LBA9).
31. Ellis MJ, Tao Y, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst* 2008; 100:1380-8.
32. Guarducci C, Bonechi M, Boccalini G, et al. mechanisms of resistance to cdk4/6 inhibitors in breast cancer and potential biomarkers of response. *Breast Care (Basel)* 2017; 12:304-8.
33. Pandey K, An HJ, Kim SK, et al. Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: a review [e-pub ahead of print]. *Int J Cancer*. https://doi.org/10.1002/ijc.32020. Accessed: December 07, 2017.
34. Guarducci C, Bonechi M, Benelli M, et al. Cyclin E1 and Rb modulation as common events at time of resistance to palbociclib in hormone receptor-positive breast cancer. *NPJ Breast Cancer* 2018; 4:38.
35. Herrera-Abreu MT, Palafox M, Asghar U, et al. Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. *Cancer Res* 2016; 76:2301-13.
36. Dean JL, Thangavel C, McClendon AK, et al. Therapeutic CDK4/6 inhibition in breast cancer: key mechanisms of response and failure. *Oncogene* 2010; 29:4018-32.
37. Arnedos M, Bayar MA, Cheaib B, et al. Modulation of Rb phosphorylation and antiproliferative response to palbociclib. The Preoperative-Palbociclib (POP) randomized clinical trial. *Ann Oncol* 2018; 29:1755-62.
38. Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res* 2010; 16:5222-32.
39. Björhle J, Bergqvist J, Gronowitz JS, et al. Serum thymidine kinase activity compared with CA 15-3 in locally advanced and metastatic breast cancer within a randomized trial. *Breast Cancer Res Treat* 2013; 139:751-8.
40. Bonechi M, Galardi F, Biagioni C, et al. Plasma thymidine kinase-1 activity predicts outcome in patients with hormone receptor positive and HER2 negative metastatic breast cancer treated with endocrine therapy. *Oncotarget* 2018; 9:16389-99.
41. Bagegni N, Thomas S, Liu N, et al. Serum thymidine kinase 1 activity as a pharmacodynamic marker of cyclin-dependent kinase 4/6 inhibition in patients with early-stage breast cancer receiving neoadjuvant palbociclib. *Breast Cancer Res* 2017; 19:123.
42. Finn RS, Dering J, Conklin D, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res* 2009; 11:R77.
43. Malorni L, Piazza S, Ciani Y, et al. A gene expression signature of retinoblastoma loss-of-function is a predictive biomarker of resistance to palbociclib in breast cancer cell lines and is prognostic in patients with ER positive early breast cancer. *Oncotarget* 2016; 7:68012-22.
44. Hurvitz S, Martin M, Wijayavardana S, et al. *Markers of response to CDK4 & 6 inhibition from neoMONARCH: a phase II neoadjuvant study of abemaciclib in postmenopausal women with hormone receptor positive, HER2 negative breast cancer*. San Antonio, TX: Presented at the San Antonio Breast Cancer Symposium; 2018, December 6-8.