CDK4/6 inhibitors in advanced breast cancer, what is beyond?

Amrallah A. Mohammed,1,2 Hanaa Rashied,3 Fifi Mostafa Elsayed4

1Medical Oncology Department, Faculty of Medicine, Zagazig University, Egypt; 2King Salman Armed Forces Hospital, Tabuk City, KSA; 3El-mabra Hospital, Zagazig, Egypt; 4Clinical Oncology & Nuclear Medicine, Department Faculty of Medicine Suez Canal University, Egypt

Abstract

Resistant to hormonal treatment considered the main clinical challenge in the management of advanced breast cancer (ABC). The use of CDK4/6 inhibitors (CDK4/6I) may change the treatment landscape. In this mandated review, we will focus on the applicable role of CDK4/6I in the management of HR+/HER2-ABC, mechanisms of resistance, and promising future implementation.

Introduction

Worldwide, breast cancer (BC) is the most common cancer in women and a major cause of disease related death. In 2018, 266,120 diagnose accounting for approximately 15% of all newly diagnosed cancers.1

Hormone receptor-positive (HR+)/HER2-BC is the most frequently diagnosed molecular subtype.2 Nearly, 6% of patients diagnosed with metastatic stage, and about half of patients with primary BC will progress later to the metastatic stage. While metastatic breast cancer (MBC) is treatable, still incurable and the aim of treatment is a palliative intent.3

The five-year relative survival (all races-females) was 98.7%, 85.3%, and 27% for localized, regional, and metastatic stages, respectively. Currently, to improve the prognosis, the recent American Joint Committee on Cancer (AJCC) incorporated the biomarkers (ER/PgR expression, HER2 status, and multigene assays) into BC staging.4

The resistance either primary or secondary with the heterogeneity of BC represented the main causes responsible for the treatment failure.

Cancer is an uncontrolled growth ultimately developed when apoptosis (programmed cell death) is broken down with a loss of cell cycle control as well as overexpression of growth signals.

In normal tissues, the cyclins; regulatory proteins activate CDKs (Cyclin-Dependent Kinases) forming complexes that regulate the cell progression from one phase to another one.

The Retinoblastoma (Rb) protein, a tumor suppressor has an axial role in the negative controls of excessive cell growth and in tumor progression depending on the phosphorylation status.5 CDKs are the main responsible for this phenomenon. It is settled that, changes in the Rb pathway have been implicated in many types of cancer. However, the rarity of the Rb gene mutation itself makes the inhibition of CDKs activity is more practical.

Currently, it has established the importance of cyclin D-CDK4/6 in BC development, and the clinical implication of therapeutic strategy.6

The role of RB-cyclin D1–CDK4/6–pathway in BC

The way of it the phosphorylation of Rb stimulates the cell proliferation had been extremely evaluated. The Rb protein presented in either phosphorylated or unphosphorylated form. When in the latter form and through the combination with an inhibition of the E2F transcription factor, it suppresses the cellular proliferation. Whereas the phosphorylation of Rb encountered by binding of cyclin D1 with CDK4/6 and stabilized by p21 protein leads to the release of the E2F with subsequent stimulation of cell cycle progression. Moreover, cyclin E protein or its encoding CCNE1 gene in combination with CDK2 leads to hyperphosphorylation of Rb releasing more E2F transcription factor and favoring the expression of a wide diversity of genes that encourage more cellular progression.7

Various mechanisms contributed to the regulation of CDK4/6 activity, included tyrosine kinases receptors (EGFR and HER2), the oestrogen receptor (ER), and the PI3K–AKT–mTOR. In a mouse model, cyclin D1 is pivotal for mammary adenocarcinomas initiation, and when combined with CDK4 enhances tumor growth. Moreover, cyclin D1 is a target of the ER, so oestrogens encourage the transition of ER+ BC cells from G1 to S phase. Furthermore, it can also promote ER expression target genes independent of oestrogen. Besides, there is inclusive crosstalk between the CDK4/6 and PI3K pathways; PI3K pathway activity increases cyclin D1 levels and D–CDK4/6 modulate mTORC1 activity by changing TSC2 phosphorylation8-10 (Figure 1).
We do not have only stimulator mechanisms responsible for the increased CDK4/6 activity, but also we have endogenous inhibitors. The INK4 proteins (p16INK4a, p15INK4b, p18INK4c, and p19INK4d) represent the most common endogenous inhibitors. In a subset of BC, patients exhibited a deletion of CDKN2A, which is the gene encoding p16. Theoretically, such patients could have higher CDK4/6 activity and so potentially be more susceptible to CDK4/6I.\textsuperscript{11}

The current practice of CDK4/6I in the treatment of HR+/HER2-ABC

Early clinical trials experienced with pan-CDKI (alvocidib and seliciclib) showed a narrow therapeutic index resulting in toxicities at the doses which sufficient to inhibit CDKs.

CDK4/6I approved efficacy in clinical and preclinical evaluation in HR+/HER2--ABC, and the best response achieved when combined with endocrine therapy (ET). Based on the improvement in the progression-free survival (PFS) in HR+/HER2-ABC, the

Figure 1. The cyclin D1-CDK4/6-RB pathway and crosstalk in breast cancer.

The ERBB2/PI3K/AKT/mTOR and ER transcription amend the levels of cyclin D1, activating CDK4/6 and enhancing cellular transition to the S phase. There is cross talk between the cyclin D1–CDK4/6-RB pathway and other signals pathways; PI3K pathway activity increase cyclin D1 levels and D–CDK4/6 modulate mTORC1 activity by changing TSC2 phosphorylation.

TKR, tyrosine kinase receptor; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; ER, estrogen receptor; mTOR, mammalian target of rapamycin; TSC2, tuberous sclerosis complex 2; RB, retinoblastoma protein; PI3K, phosphoinositide-3-kinase; S6K1, Ribosomal protein S6 kinase beta-1.
U.S. Food and Drug Administration (FDA) approved three CDK4/6I in combination with ET (except abemaciclib can be used as a single agent). Each one of them has different pharmacokinetics, potency, dosing and toxicity profiles. Table 1 summarizes the clinical trials that led to the FDA approvals for CDK4/6I in HR+/HER2-ABC (first-line and post-ET).

### Palbociclib

**PALOMA-1** is a phase II trial included 165 post-menopausal women with ER+/HER2-ABC with no prior systemic therapy. They were randomized to receive either palbociclib 125 mg for 3 weeks on and one week off with letrozole 2.5 mg/daily (n=84) or letrozole alone 2.5 mg/daily (n=81). Patients with more than 12 months’ treatment with an aromatase inhibitor (AI) were included in the study. The median PFS was 20.2 months for palbociclib/letrozole while it was 10.2 months for letrozole alone. There was a numerical OS benefit of the combination arm versus single one (37.5 m vs 34.5 m), (HR, 0.897; 95% CI, 0.623-1.294; P=0.281). The study was not powered to clarify the OS difference.13,14

**PALOMA-2** is a phase III trial with the same design as PALOMA-1. The median PFS for the combined arm (palbociclib/letrozole) was 24.8 months and was 14.5 months for letrozole alone.15

**PALOMA-3**, patients with HR+/HER2-ABC that relapsed within 12 months of stopping adjuvant ET or progressed during it, any menopausal status, ≤1 line of chemotherapy for advanced disease (N=521) were randomized to receive either palbociclib 125 mg for 3 weeks on and one week off with fulvestrant 500 mg IM/14 day for the first 3 injections then /28 day (n=347) or single-agent fulvestrant 500 mg IM/14 day for the first 3 injections then /28 day (n=174). The final results showed a median PFS was 9.5 months and was 4.5 months in the palbociclib/fulvestrant arm and fulvestrant alone, respectively.16,17

### Ribociclib

**MONALEESA-2** is a phase III trial evaluated letrozole/ribociclib or letrozole alone in the first-line treatment in patients with HR+/HER2-ABC. Final analysis revealed PFS was 25.3 months for the combined group compared with 16 months in the single-arm group.18,19

**MONALEESA-3** is another phase III randomized trial to assess the use of ribociclib combined with fulvestrant in the first-line and second-line treatment of HR+/HER2-ABC. The inclusion criteria were post-menopausal women with HR+/HER2-ABC, with/without previous one line of an ET for advanced disease (N=726). They were randomized to receive either ribociclib 600 mg/day for 3 weeks on and one week off with fulvestrant 500 mg IM on Days 1, 15 of cycle 1 then on day 1 of 28-day cycles (n=484) or placebo +fulvestrant 500 mg IM on days 1, 15 of cycle 1 then on day 1 of 28-day cycles (n=242). The final report demonstrated a median

---

**Table 1. Summarizes the clinical trials that led to the FDA approvals of CDK4/6I in HR+/HER2–MBC.**

| Trials name   | N    | Phase | Descriptions                  | ORR   | PFS HR (95% CI) |
|---------------|------|-------|--------------------------------|-------|----------------|
| PALOMA-1      | 165  | II    | Palbociclib/Letrozole          | 55    | 20.2 m vs 10.2 m |
|               |      |       | *vs*                           | 39    | 0.49 (0.32 0.75) |
| PALOMA-2      | 666  | III   | Palbociclib/Letrozole          | 55    | 24.8 vs 14.5    |
|               |      |       | *vs*                           | 44    | 0.58 (0.46-0.72) |
| MONALEESA-2   | 668  | III   | Ribociclib/Letrozole           | 53    | 25.3 vs 16      |
|               |      |       | *vs*                           | 37    | 0.56 (0.43-0.72) |
| MONARCH-3     | 493  | III   | Abemaciclib/AI                 | 59    | 28.1 vs 14.8    |
|               |      |       | *vs*                           | 44    | 0.54 (0.41-0.72) |
| MONALEESA-7   | 672  | III   | Ribociclib/OFS/AI or TAM       | 51    | 23.8 vs 13      |
|               |      |       | *vs*                           | 36    | 0.55 (0.44-0.69) |

**Post-endocrine treatment**

| Trials name   | N    | Phase | Descriptions                  | ORR   | PFS HR (95% CI) |
|---------------|------|-------|--------------------------------|-------|----------------|
| PALOMA-3      | 521  | II    | Palbociclib/Fulvestran         | 25    | 9.5 vs 4.6     |
|               |      |       | *vs*                           | 11    | 0.46 (0.36-0.59) |
| MONALEESA-3   | 752  | III   | Ribociclib/Fulvestran          | 40.9  | 20.5 vs 12.8   |
|               |      |       | *vs*                           | 28.7  | 0.59 (0.48-0.73) |
| MONARCH-2     | 669  | III   | Abemaciclib/Fulvestran         | 48    | 16.4 vs 9.3    |
|               |      |       | *vs*                           | 21    | 0.55 (0.45-0.68) |
| MONARCH-1     | 132  | II    | Abemaciclib/Monotherapy        | 20    | 6.0            |

CDK4/6I, cyclin-dependent kinases 4/6 inhibitors; HR+, hormone receptors positive; MBC, metastatic breast cancer; N, number of patients; ORR, objective response rate; PFS, progression free survival; OS, overall survival; OFS, ovarian function suppression; AI, aromatase inhibitors; TAM, tamoxifen. Palbociclib, dose: 125 mg/day, 3 weeks on/one week off, half-life, 27 h; Ribociclib, dose: 600 mg/day, 3 weeks on/one week off, half-life, 36.5 h; Abemaciclib, dose, monotherapy: 200 BID, combined: 150 BID, continuous, half-life, 17.38 h.
PFS in the ribociclib/fulvestrant arm was 20.5 months, compared with 12.8 months in the placebo/fulvestrant arm.\(^{20}\)

**Abemaciclib**

The most recently approved CDK4/6I in the treatment of HR+/HER2-ABC either in the first line or the second-line. It is the only one that can be used either in combination with ET or as a monotherapy, referring to MONARCH serial trials.

MONARCH-3 is a phase III trial evaluated either AI/abemaciclib or AI/ placebo as a first-line treatment in patients with HR+/HER2-ABC. The final analysis demonstrated that PFS was 14.8 months for AI alone. Whereas, in the combined group the PFS was 28.1 months.\(^{21,22}\)

MONARCH-2 is a phase III trial evaluated fulvestrant alone or combined with abemaciclib. The inclusion criteria were patients with HR+/HER2-ABC that had progressed during prior ET, any menopausal status, ≤1 ET, no prior chemotherapy for advanced disease (N=669). They were randomized either to abemaciclib 200 mg every 12 hours/fulvestrant 500 mg IM on days 1, 15 of cycle 1 then day 1 of 28-day cycles (n=446) or placebo/fulvestrant 500 mg IM on days 1, 15 of cycle 1 then day 1 of 28-day cycles (n=223). The PFS was 14.4 months and was 9.3 months for the combined group and placebo group, respectively.\(^{21}\)

MONARCH-1 is a phase II trial assessed single-agent abemaciclib (200 mg every 12 hours until unacceptable toxicity or disease progression) in patients with HR+/HER2-ABC with progression on or after prior ET. After 12 months follow up period, the clinical benefit rate was 42.4% and confirmed overall response rate was 19.7%.\(^{24}\)

**CDK4/6I in premenopausal status**

The rationale for the use in the premenopausal setting is based on previous data denoting the benefit from ovarian function suppression (OFS) in patients with HR+/HER2-ABC. MONALEESA-7 is the first phase III trial evaluated the use of ribociclib vs placebo with goserelin/AI or tamoxifen as first-line in premenopausal status. The final analysis demonstrated that the improvement in PFS was comparable to that saw in MONALEESA-2 in postmenopausal patients. Although in MONARCH-1 the included patients were regardless of the menopausal state, they were after more than one line of chemotherapy, so presumably, they have some degree of ovarian failure.

**Safety and efficacy of CDK4/6I in HR+/HER2-ABC**

Generally, there were no safe medications, but CDK4/6I are drugs with an acceptable and well tolerable side effect. There is no major advantage of one drug over the other, it is a patient-physician choice. So it is accepted to try an alternative one if the patient is suffering from intolerance. Hematological, gastrointestinal manifestations and QTc prolongation are the most common adverse effects with the neutropenia most commonly observed with palbociclib and ribociclib. Whereas diarrhea and increased serum level of creatinine are commonly associated with abemaciclib as a result of the inhibitory effect on the renal tubules. The difference in toxicity likely explained by the difference in potency in the inhibition of CDKs.\(^{25}\) Although, there was a considerable number of patients with neutropenia, the risk of febrile neutropenia or infection were not high. The difference in mechanism of action between CDKI and chemotherapeutic agents may explain this difference. The bone marrow suppression induced by CDKI occurred through a reversible cell cycle arrest. Meanwhile, the chemotherapeutic agents produce irreversible cell death through DNA damages.\(^{26}\)

Regarding the three CDK4/6I, it is preferable to start with the approved doses without initial adjustments. In the case of toxicities, we should follow guidelines of dose modification. Patients must know that they still can get a benefit even in lower doses.

Referring to the efficacy of the three approved CDK4/6I, palbociclib, ribociclib, and abemaciclib in the treatment of HR+/HER2-ABC, no head to head trials comparing them against each other, so till now no superiority of one agent over the other. It appears clear when used in the naïve cases, referring to PALOMA-1/PALOMA-2, MONALEESA-2 and MONARCH-3 trials, the prolongations of PFS are comparable.

For the use in second- or later-line treatment, still both palbociclib and ribociclib have similar results as approved in PALOMA-3 and MONALEESA-3, respectively. However, the results of MONARCH-2 seem different in comparison to PALOMA-3 (PFS in combined arms was 16.4 months and was 9.5 months, respectively). This perhaps related to the patient selection criteria. In MONARCH-2, patients received chemotherapy in a metastatic setting were ineligible, only post one line of ET, and only 59% had received an ET either in a neoadjuvant or adjuvant setting. Whereas in PALOMA-3, approximately one-third of the enrolled patients had received chemotherapy, and approximately half of the patients had received more than one line of ET in the metastatic setting, denoting that patients with treatment resistance have a poorer outcome to subsequent therapy.\(^{27}\)

However, subgroup analysis in PALOMA-3 trial proposed that the OS benefit of palbociclib confined to endocrine sensitive tumors.\(^{28}\) Moreover, in 3 pooled analyses and in the MONARCH-2 trial reported less benefit from abemaciclib in low-risk patients (e.g. bone metastasis only, long treatment interval) compared with endocrine insensitive patients (liver metastasis) who achieved more benefits.\(^{29,30}\) Notably, Asian patients had higher PFS in comparison with other ethnicities. This observation might be related to different pharmacokinetics, tolerance or efficacy to CDKI.\(^{31}\)

**Cost-effectiveness of CDK4/6I**

Although, the meaningful improvement in the PFS with the use of three CDK4/6I, palbociclib, ribociclib, and abemaciclib in HR+/HER2-ABC either in the first or second-line, the cost-effectiveness evaluation is not enough evaluated. Mistry et al. evaluated the cost-effectiveness of ribociclib plus letrozole versus palbociclib plus letrozole and versus letrozole monotherapy in the first-line treatment of HR+/HER2-ABC from a United States private third-party payer perspective. They reported that ribociclib plus letrozole is a cost-effective alternative to palbociclib plus letrozole for the first-line treatment in HR+/HER2-ABC. Ribociclib plus letrozole is also cost-effective versus letrozole monotherapy.\(^{32}\)

In contrast, recently, Zhang et al. conducted a study to evaluate the cost-effectiveness of, palbociclib or ribociclib, for the treatment of these subtypes of patients in the United States. Through the Markov simulation model, the authors concluded that the addition of palbociclib or ribociclib to letrozole in the treatment of HR+/HER2-ABC is not cost-effective in the United States given current drug prices.\(^{33}\)
The sequence of CDK4/6I

Already, we have a standard of care regimens for treatment of HR+/HER2- ABC based on ET; they included tamoxifen, selective estrogen receptor modulator (1970-1980); anastrozole, letrozole, and exemestane, AIs (1980s); fulvestrant, selective estrogen receptor down-regulated (2002), high dose fulvestrant (2010), everolimus, mTOR inhibitor (2012); palbociclib, CDK4/6I (2015-2017), ribociclib, and abemaciclib, CDK4/6I (2017-2018).

Although CDK4/6I is approved in both first-line and later, there are no clear guidelines for whom to use in the first-line or later. The majority of guidelines advice to start with CDK4/6I in HR+/HER2-ABC in the first-line setting. The addition of CDK4/6I to any line of ET yields a significant improvement in PFS compared to placebo. Figure 2 illustrates the proposed algorithm for the management of HR+/HER2-ABC.

Generally, the indolent course of HR+ ABC includes late recurrence of metastases, long disease-free interval, soft tissue, and bone disease responds better to endocrine therapy. Historically, it was not allowed to use ET in the case of visceral metastasis, however, in sub-analysis of the CDK4/6I trials demonstrated up to 65% of enrolled patients were with visceral metastasis and showed a comparable benefit that seen in all study population. However, still, the chemotherapy is the drug of choice in the visceral crisis. Although the data on OS is immature, the significant improvement in PFS is enough to delay the progression of more serious symptoms.34

![Proposed algorithm for management of HR+/HER2-ABC.](image)

- TAM, Tamoxifen; AI, Aromatase inhibitors; CDK4/6, cyclin dependent kinase inhibitors.
- Chemotherapy when they develop resistance or experience a visceral crisis.
- Aromatase inhibitors with ovarian function suppression in premenopausal patients.
- We can use abemaciclib as monotherapy in third line when the patients were CDK4/6 inhibitors naïve.
- Although the clinical trials showing fulvestrant’s efficacy in this setting were conducted prior to CDK4/6 inhibitor use.
- The efficacy of exemestane/everolimus combination after receiving a CDK4/6 inhibitor with endocrine therapy is unknown.
The next step in CDK4/6I

Molecular biomarkers and resistance to CDK4/6I-based therapy

Preclinical and clinical studies reported the presence of both de novo and acquired resistance. About 20% of the patients will experience primary resistance and all of them will eventually develop treatment failure of CDK4/6I treatment.

Preliminary mechanisms responsible for the resistance could be classified into two main categories. The first one is cell cycle components which included; loss/inactivation of Rb protein. Many trials had reported that functional and intact Rb protein is a core in CDK4/6I favoring its use as a potential biomarker. However, the loss of Rb protein was variable and dependent on the molecular subtypes with HR+ subtype probably has an intact and functional Rb pathway (less than 4% of HR+ BC with Rb deletion/mutation). So the CDK4/6-cyclin D pathway could be interrupted by different cell cycle components mechanisms, like overexpression of cyclin D1, cyclin D2, cyclin D3, cyclin E, CDK6, CCNE1 amplification, and loss/inactivation of CDK2 physiological inhibitors (p21, p27).

The second one is adaptive mitogenic signaling which involved upregulation of mTOR and PI3K/AKT. So the dual blockade of CDK4/6I with PI3K/mTOR pathway (e.g., idelalisib, everolimus) or Ras/Raf/MEK pathway (e.g., vemurafenib, binimetinib) may overcome the resistance and enhance the therapeutic effect.

In the case of de novo resistance, through the subgroup analyses in PALOMA-3 trial showed that the CCNE1 expression retained an association with benefit from palbociclib after adjusting for prognostic baseline characteristics, while cyclin E is considered as a driver of resistance to CDK4/6I. Moreover, in a subset of patients with Rb loss and cyclin E overexpression, they had shorter PFS. Also, in MONALEESA-2 trial the amplification of fibroblast growth factor receptor (FGFR1) was associated with resistance to ET and ribociclib. While in a case of acquired resistance and through sub-analysis of 195 patients from PALOMA-3 trial of fulvestrant/palbociclib or fulvestrant alone, there was no Rb mutations detection at a baseline (0/193) with truncating (single nucleotide variants or indels) mutations emerging only in patients on palbociclib (4.8%; 6/125) vs fulvestrant alone (0/68), and most of these mutations were sub-clonal.

Moreover, the frequent acquisition of new PIK3CA and ESR1 mutations were reported. Approximately, 6% of patients with no detectable PIK3CA mutations had acquired new PIK3CA mutations. Positive selection of Y537S at the end of the treatment (ligand-binding domain mutation) is highly resistant to fulvestrant.

A proper realization of molecular biomarkers may help in therapy selection to get more improvement in outcome. Currently, many trials designed to evaluate the predictive biomarkers to CDK4/6I therapy at protein and gene levels (PYTHIA [NCT02536742, NCT03195192; (NCT03195192). PROMISE is a prospective study evaluating blood (circulating tumor cells and circulating tumor DNA) and tumor sequence. The role of the microbiome and the development of patient-derived xenograft models to identify biomarkers of response to palbociclib and ET for HR+/HER2-ABC (ClinicalTrials.gov ID: NCT03281902). Another two ongoing phases I trials including palbociclib with either T-DM1 (NCT01976160) or with paclitaxel (NCT01320592) had included the Rb expression in the inclusion criteria. With the lack of acceptable biomarkers for CDK4/6I sensitivity, investigators are currently evaluating the signatures of sen-
sitivity. For example, a gene expression signatures of inactive CDK4 and in Rb loss deduce from E2F1 and E2F2. Currently, eleven gene expression signatures are being evaluated and validated in the neoadjuvant before and after four cycles of palbociclib and ET (NeoRHEA trial (Nbib306521)).

**CDK4/6I with PI3K inhibitors in PIK3CA mutant tumors**

Many trials to overcome this resistance. The synergistic effect of CDK4/6I with PI3K pathway inhibitors had been investigated in and mTORC1 activity, inhibiting the two main stimulators of S phase progression. The crosstalk between cyclin D1–CDK4/6–RB pathway and other signaling pathways, the combo inhibition of both the PI3K pathway and c-myc, results in the suppression of the RB phosphorylation. CDK4/6I will act synergistically suppressing Rb phosphorylation and mTORC1 activity, inhibiting the two main stimulators of S phase progression. The synergistic effect of CDK4/6I with PI3K inhibitors in PIK3CA mutant tumors may explain the strategy of the combination.

However, the multiple mechanisms mediating antitumor effects (proliferation, immune effects, and metastases) propose the continuation of CDK4/6I after the first progression.32 Based on the crosstalk between cyclin D1–CDK4/6–RB pathway and other signals pathways, the combo inhibition of both the PI3K pathway and CDK4/6I will act synergistically suppressing Rb phosphorylation and mTORC1 activity, inhibiting the two main stimulators of S phase progression. The triple therapy combination of ET and CDK4/6I with PI3K pathway inhibitors had been investigated in many trials to overcome this resistance. The synergistic effect of CDK4/6I with PI3K inhibitors in PIK3CA mutant tumors may explain the strategy of the combination.

Recently, FDA approved alpelisib (alpha-specific PI3KI) for HR+/HER2-ABC, PIK3CA-mutated after the failure of ET. The approval based on SOLAR-1 phase III randomized trial. The inclusion criteria were postmenopausal women/ men with HR+/HER2-ABC after ≥ 2 lines of ET without chemotherapy. They randomized to oral alpelisib (300 mg/day) or placebo plus fulvestrant (500 mg/28 days on days 1 and 15 of treatment cycle 1) (about 6% had received prior CDK4/6 therapy). There was a 7.4-month improvement in the alpelisib arm compared with the placebo arm (the median PFS was 11.1 months versus 3.7 months, respectively).44 In addition, the inhibition of E2F activity may change the tumor epigenome making the tumor more immunogenic (T-cell activation, enhance tumor antigen presentation and suppressing proliferation of regulatory T-cells), that give a rationale for immunotherapy (targeting cytotoxic T-lymphocyte associated protein 4 or Programmed cell death protein-1) – CDK4/6I combinations.45 The results of a phase I trial from mouse model HR+/HER2- included abemaciclib with pembrolizumab reported at a 16-week interim analysis an overall response rate of 14.3% with accepted safety profiles.46 Of note, FGFR inhibitor/CDK4/6I, oral SERDs and SERMs (e.g., endoxifen, lasofoxifene) targeting ESR1 mutations, and other pathways driving CDK4/6I resistance is valid examples combinations currently under investigations. Table 2 summarized the ongoing trials of CDK4/6I-based treatment after progression of HR+/HER2-ABC.

**Moving CDK4/6I into earlier stages**

BC recurrence risk remains high among women with early-stage ER+ BC who were disease-free after 5 years of ET (17% to 26% through 20 years).47 Owing to the meaningful improvement in PFS with the use of CDK4/6I in HR+/HER2+ ABC, ongoing phase II/III trials to address the value of adding them to ET in the adjuvant/neoadjuvant setting. The early results from phase II trials revealed that CDK4/6I enhanced anti-proliferative activity in ER+ EBC. In NeoPalAna trial, 87% complete cell cycle arrest with palbociclib/anastrozole compared to 26% with anastrozole only (P<0.001.48 Meanwhile, in MONALEESA-1 trial, the mean decrease in Ki-67+ cells was 92% and was 69% for ribociclib/letrozole and letrozole alone, respectively.49 Table 3 shows some ongoing trials in early-stage ER+/HER2-ABC.

**CDK4/6I for HR+/HER2+ABC**

There is some data suggested that the CDK4/6I may show some benefit of HR+/HER2+ABC. Since many ongoing phase II and III trials to justify the role of CDK4/6I with anti-HER2 therapy in HR+/HER2+ABC; NCT02448420, palbociclib, and trastuzumab+letrozole; NCT02947685, anti-HER-2 therapy/ET+pallbociclib; NCT02657343, ribociclib in combination with trastuzumab or T-DM1.

### Table 2. Ongoing trials of CDK4/6I–based therapy beyond progression in HR+/HER2–MBC.

| Trials identifier | Design | Planned N. | Phase | Primary end point |
|-------------------|--------|------------|-------|-------------------|
| MAINTAIN (NCT02632045) | Ribociclib + fulvestrant vs placebo + fulvestrant | 132 | II | PFS |
| NCT02871791 | Palbociclib + everolimus + exemestane | 32 | II | DLTs, CBR |
| NCT01857193† | Ribociclib + exemestane ± everolimus | 132 | I | DLTs, safety and tolerability |
| TRINITI-I (NCT02732119) | Ribociclib + everolimus + exemestane | 51 | II | Phase I: MTD/RP2D Phase II: CBR |
| PACE (NCT03147287) | Fulvestrant vs fulvestrant + palbociclib ± avelumab | 220 | II | PFS |
| NCT02738866 | Palbociclib + fulvestrant | 100 | II | PFS, prevalence of ESR1 and PI3K mutations |

**Notes:** Trials identifier, ClinicalTrials.gov. Identifier; N, number of patients; MBC, metastatic breast cancer; CDK4/6I, Cyclin-Dependent Kinases 4/6 inhibitors; HR, hormone receptor positive; CBR, clinical benefit rate; DLT, dose limiting toxicity; HR, hormone receptor; MTD, maximum tolerated dose; PFS, progression free survival; RP2D, recommended phase II dose; PI3K, Phosphoinositide 3-kinase; ESR1, estrogen receptor 1.

### Table 3. Ongoing trials in early-stage HR+/HER2- BC.

| Trials identifier | Patients types | Treatment plan |
|-------------------|----------------|----------------|
| PALLAS (NCT02513394) | Stage II-III invasive BC | SoC adjuvant ET ± palbociclib |
| PENELLOPE-B (NCT01864746) | Residual disease post neoadjuvant CT, high relapse risk | SoC adjuvant ET ± palbociclib |
| MonarchE (NCT03155997) | High-risk, node-positive BC post-surgery | SoC adjuvant ET ± abemaciclib |
| NATALEE (NCT03078751) | Stage II-III invasive BC | SoC adjuvant ET ± ribociclib |

**Notes:** Trials identifier, ClinicalTrials.gov. Identifier; SoC, standard of care; CT, chemotherapy; ET, endocrine therapy; BC, breast cancer.
Conclusions

For HR+/HER2-ABC, the treatment landscape had been changed by introducing CDK4/6i. The three approved drugs; palbociclib, ribociclib, and abemaciclib are equally effective. No one recommends detaining any therapy with a better outcome to be used in second-line. CDK4/6i should be initiated into full dose and adjusted as needed for toxicities. The accepted side effect and the good tolerability makes their use for the elderly are a valid option. Although the extensive research, the ER+ remains the best predictive biomarker for response to CDK4/6i. The chance of utilizing biomarkers to predict the outcome, novel therapy combinations and the possible activity of CDK4/6i beyond the use in HR+/HER2-ABC are fields of dynamic research.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:70-130.
2. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 2014;28:106.
3. Brufsky AM. Long-term management of patients with hormone receptor-positive metastatic breast cancer: Concepts for sequential and combination endocrine-based therapies. Cancer Treat Rev 2017;59:22-32.
4. Hortobagyi GN, Edge SB, Giuliano A. New and important changes in the TNM staging system for breast cancer. Am Soc Clin Oncol Educ Book 2018;23:457-67.
5. Burkhardt DL, Sage J. Cellular mechanism of tumor suppression by the retinoblastoma gene. Nat Rev Cancer 2008;8:671-82.
6. Stone A, Sutherland RL, Musgrove EA. Inhibitors of cell cycle kinases: recent advances and future prospects as cancer therapeutics. Crit Rev Oncog 2012;17:175-98.
7. Narasimha AM, Kaulich M, Shapiro GS, et al. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. Elife 2014;3.
8. Goel S, Wang Q, Watt AC, et al. Overcoming therapeutic resistance in HER2-positive breast cancers with CDK4/6 inhibitors. Cancer Cell 2016;29:255-69.
9. Vora SR, Juric D, Kim N, et al. CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell 2014;26:136-49.
10. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nat Rev Cancer 2017;27:93-115.
11. Cerami E, Gao J, Dogrusoz U, et al. The eBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.
12. Shah M, Nunes MR, Stearns V. CDK4/6 inhibitors: game changers in the management of hormone receptor-positive advanced breast cancer? Oncology 2018;32:216-22.
13. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus 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;16:235-35.
14. Finn RS, Crown J, Lang I, et al. Overall survival results from the randomized phase II study of palbociclib (P) in combination with letrozole (L) vs letrozole alone for frontline treatment of ER+/HER2- advanced breast cancer (PALOMA-1; TRIO-18). J Clin Oncol 2017;35:abstr 1001.
15. Finn RS, Martin M, Rugo HS, et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med 2016;375:1925-36.
16. Turner NC, Ro J, Andre F, et al. Palbociclib in hormone-receptor-positive advanced breast cancer. N Engl J Med 2015;373:209-19.
17. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus 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, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016;17:425-39.
18. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as first-line therapy for HR-positive, advanced breast cancer. N Engl J Med 2016;375:1738-48.
19. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase 3 trial of first-line ribociclib + letrozole in hormone receptor-negative (HR-), HER2-negative (HER2-), advanced breast cancer (ABC). Ann Oncol 2018;29:1541-7.
20. 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.
21. 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.
22. di Leo A, Toi M, Campone M, et al. MONARCH 3: Abemaciclib as initial therapy for patients with HR+/HER2-advanced breast cancer. Ann Oncol 2017;28:605-49.
23. 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.
24. Dickler MN, Tolaney SM, Rugo HS, et al. MONARCH 1, a phase II study of abemaciclib, a CDK4 and CDK6 inhibitor, as a single agent, in patients with refractory HR(+)/HER2(-) metastatic breast cancer. Clin Cancer Res 2017;23:5218-24.
25. Costa R, Gradishar WJ. Differences are important: breast cancer therapy in different ethnic groups. J Glob Oncol 2017;3:281-4.
26. Hu W, Sung T, Jessen BA, et al. Mechanistic investigation of bone marrow suppression associated with palbociclib and its differentiation from cytotoxic chemotherapies. Clin Cancer Res 2016;22:2000-8.
27. Condorelli R, Spring L, O’Shaughnessy J, et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol 2018;1:29:640-645.
28. Turner NC, Slamon DJ, Ro J, et al. Overall survival with palbociclib and fulvestrant in advanced breast cancer. N Engl J Med 2018;379:1926-36.
29. di Leo A, O’Shaughnessy J, Sledge GW Jr, et al. Prognostic characteristics in hormone receptor-positive advanced breast cancer and characterization of abemaciclib efficacy. NPJ Breast Cancer 2018;4:41.
30. O’Shaughnessy J, Goetz MP, George W, et al. The benefit of abemaciclib in prognostic subgroups: An update to the pooled analysis of MONARCH 2 and 3. Cancer Res 2018;78:abstr CT099.
31. Barroso-Sousa R, Shapiro GI, Tolaney SM. Clinical develop-
ment of the CDK4/6 inhibitors ribociclib and abemaciclib in breast cancer. Breast Care (Basel) 2016;11:167-73.

32. Mistry R, May JR, Suri G, et al. Cost-effectiveness of ribociclib plus letrozole versus palbociclib plus letrozole and letrozole monotherapy in the first-line treatment of postmenopausal women with HR+/HER2-advanced or metastatic breast cancer: a U.S. payer perspective. J Manag Care Spec Pharm 2018;24:514-23.

33. Zhang B, Long EF. Cost-effectiveness analysis of palbociclib or ribociclib in the treatment of advanced hormone receptor-positive, HER2-negative breast cancer. Breast Cancer Res Treat 2019;175:775-9.

34. Wilcken N, Hornbuckle J, Ghersi D. Chemotherapy alone versus endocrine therapy alone for metastatic breast cancer. Cochrane Database Syst Rev 2003;CD002747.

35. Konecny GE, Winterhoff B, Kolarova T, et al. Expression of p16 and retinoblastoma determines response to CDK4/6 inhibition in ovarian cancer. Clin Cancer Res 2011;17:1591-602.

36. Cirriello G, Gatza ML, Beck AH, et al. Comprehensive molecular portraits of invasive lobular breast cancer. Cell 2015;163:506-19.

37. Turner NC, Yuan Liu Y, Zhou Z, et al. Cyclin E1 (CCNE1) expression associates with benefit from palbociclib in metastatic breast cancer (MBC) in the PALOMA3 trial. Cancer Res 2018;78:abstr CT039.

38. Vijayaraghavan S, Karakas C, Doostan I, et al. CDK4/6 and autophagy inhibitors synergistically induce senescence in Rb positive cytoplasmic cyclin E negative cancers. Nat Commun 2017;8:15916.

39. Formisano L, Stauffer KM, Young CD, et al. Association of FGFR1 with ESR1 maintains ligand-independent ER transcription and mediates resistance to estrogen deprivation in ER+ breast cancer. Clin Cancer Res 2017;23:6138-50.

40. O’Leary B, Cutts RJ, Liu Y, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 2018;8:1390-403.

41. Knudsen ES, Witkiewicz AK. The strange case of CDK4/6 inhibitors: mechanisms, resistance, and combination strategies. Trends Cancer 2017;3:39-55.

42. Dhakal A, Matthews CM, Levine EG, et al. Efficacy of palbociclib combinations in hormone receptor-positive metastatic breast cancer patients after prior everolimus treatment. Clin Breast Cancer 2018;18:e1401-5.

43. Preusser M, De Mattos-Arruda L, Thill M, et al. CDK4/6 inhibitors in the treatment of patients with breast cancer: summary of a multidisciplinary round-table discussion. ESMO Open 2018;3:e000368.

44. André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019;380:1929-40.

45. Pernas S, Tolaney SM, Winer EP, Goel S. CDK4/6 inhibition in breast cancer: current practice and future directions. Ther Adv Med Oncol 2018;17.

46. Rugo H, Kabos, Dickler M, et al. Abstract P1-09-01: A phase 1b study of abemaciclib plus pembrolizumab for patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (MBC). Cancer Res 2018;78:P1-09-01.

47. Pan H, Gray R, Braybrooke J, et al. EBCTCG. 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N Engl J Med 2017;377:1836-46.

48. 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.

49. Curigliano G, Gómez Pardo P, Meric-Bernstam F, et al. Ribociclib plus letrozole in early breast cancer: a presurgical, window-of-opportunity study. Breast 2016;28:191-8.

50. Huang HW, Huang LS, Xu QN, et al. CDK4/6 inhibition versus mTOR blockade as second-line strategy in postmenopausal patients with hormone receptor-positive advanced breast cancer: a network meta-analysis. Medicine 2019;98:e13909.