Hormonoresistance in advanced breast cancer: a new revolution in endocrine therapy

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Abstract: Endocrine therapy is the mainstay of treatment of estrogen-receptor-positive (ER+) breast cancer with an overall survival benefit. However, some adaptive mechanisms in the tumor emerge leading to the development of a resistance to this therapy. A better characterization of this process is needed to overcome this resistance and to develop new tailored therapies. Mechanisms of resistance to hormone therapy result in activation of transduction signal pathways, including the cell cycle regulation with cyclin D/CDK4/6/Rb pathway. The strategy of combined hormone therapy with targeted agents has shown an improvement of progression-free survival (PFS) in several phase II or III trials, including three different classes of drugs: mTOR inhibitors, PI3K and CDK4/6 inhibitors. A recent phase III trial has shown that fulvestrant combined with a CDK 4/6 inhibitor doubles PFS in aromatase inhibitor-pretreated postmenopausal ER+ breast cancer. Other combinations are ongoing to disrupt the interaction between PI3K/AKT/mTOR and cyclin D/CDK4/6/Rb pathways. Despite these successful strategies, reliable and reproducible biomarkers are needed. Tumor genomics are dynamic over time, and blood-based biomarkers such as circulating tumor DNA represent a major hope to elucidate the adaptive mechanisms of endocrine resistance. The optimal combinations and biomarkers to guide this strategy need to be determined.

Keywords: adaptive mechanism, CDK4/6 inhibitor, hormonoresistance, PI3K/mTOR inhibitor

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Introduction

The estrogen receptor (ER) pathway is directly or indirectly activated by estrogen leading to proliferation, differentiation, invasion and cell survival. This pathway is considered an addictive oncogenic pathway in breast cancer cells. In frontline therapy, response rates to anti-estrogenic agents range from 20 to 40%, with a median duration of response of 14 months. In second-line treatment, resistance to anti-estrogenic agents is relatively high as the response rates are less than 10%, with a median progression-free survival of around 4 months.

There is no clear definition of the resistance to hormone therapy (HT). ESO–ESMO guidelines define primary endocrine resistance as: relapse while on the first 2 years of adjuvant endocrine therapy (ET) or progression of disease (PD) within the first 6 months of first-line ET for metastatic breast cancer. Secondary (acquired) resistance is defined as relapse while on adjuvant ET but after the first 2 years, or relapse within 12 months of completing adjuvant ET, or PD 6 months after initiating ET for metastatic breast cancer (MBC).1

The expression of ER is not sufficient to identify patients who will respond to anti-estrogenic therapy. Several adaptive mechanisms of escape to anti-estrogenic therapy have been identified: an increase in concentration of estrogen in the tumor.
environment, post-translational modifications, ligand-independent activation of the receptor via ESR1 gene mutation, amplification of the feedback loops mediated by transmembrane growth factor receptors [human epidermal growth factor receptor (HER), fibroblast growth factor receptor (FGF-R), insulin growth factor receptor (IGF-R)], by the RAS/rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase (MAPKinase) pathway and the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway.2 Downstream, hormone therapy resistance could be characterized by deregulation of the cell cycle involving the cyclin D/CDK4/6/Rb pathway.3 Several combination strategies with HT have failed in advanced breast cancer such as that combining EGFR inhibitors (gefitinib) and IGFR 1 inhibitors (ganitumumab).4,5 Other combinations with a SRC (rous sarcoma protein) inhibitor (dasatinib), an antiapoptotic inhibitor (bortezomib) or with histone deacetylase inhibitors (HDAC inhibitors) have shown discordant or interesting responses and need more investigations (Table 1).6–8

We hypothesize that addition is more efficient than a substitution strategy for the treatment of endocrine-resistant MBC, as shown in the HER2+ MBC context.9 We will review randomized phase II and III clinical trials exploiting this strategy.

The proof of concept of adaptive mechanisms with PI3K/Akt/mTOR inhibitors

The PI3K/Akt/mTOR pathway plays a key role in cell signaling, regulating proliferation, survival and differentiation.10 The PI3K proteins are kinases divided into three classes (I-III) according to their structure and substrate specificity.

Aberrant activation of the PI3K/Akt/mTOR pathway plays a major role in the mechanisms of resistance to HT. It is a prime target for the treatment of ER-positive breast cancers, the aim being to prevent and revert resistance to HT.11–13 The IGF/IGF-1-IRS pathway (insulin receptor substrate 1) induces activation of PI3K and activates mTORC1 with an indirect correlation between PI3K and the mTOR1 effector. Inactivation of PI3K induces inhibition of S6K1 and 4E-BP, and PI3K activation is negatively regulated by the tumor suppressor gene PTEN (phosphatase and tensin counterpart deleted one chromosome ten). The main effector of PI3K is AKT.14,15 This serine/threonine kinase belongs to the family of AGC kinases and exists in three isoforms, Akt-1, 2, 3, encoded by three different genes. Activated AKT induces the activation of the mTOR pathway promoting cell proliferation and inducing inhibition of proapoptotic proteins. mTOR was identified in the yeast Saccharomyces cerevisiae as a therapeutic target of the macrolide antibiotic, rapamycin.16 It plays a key role in the

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**Table 1.** Trials with endocrine therapy resistance (without PI3K/Akt/mTOR or CDK4/6/Rb pathways inhibition).

| Reference            | Target/pathway | Results | PFS        |
|----------------------|----------------|---------|------------|
| Paul et al., 2013    | Dasatinib      | Phase IIR First line | Let: 9.9 months |
|                      | (Inhibitor of SRC) | n = 120 | Let + dasa: 20.1 months |
| Yardley et al., 2013 | Entinostat     | Phase IIR Second line | Exe 2.3 months |
|                      | (Inhibitor of HDAC) | n = 130 | Exe + enti: 4.3 months |
| Trifonidiseur et al., 2016 | Gefinitib | Phase IIR First line | Ana + gef: 35% [at 1 year] |
|                      | Anti HER1      | n = 71  | Ana: 32% [at 1 year] |
| Robertson et al., 2013 | Ganimutab     | Phase IIR | Ful ou exe + gan: 3.9 months |
|                      | Anti IGF1      | n = 156 | Ful ou exe: 5.7 months |
| Kaufmann et al., 2009 | HER 2         | Phase III, First line | Ana + trast: 4.8 months versus |
|                      |                | n = 207 | Ana: 2.4 months |
| Jonhston et al., 2009 | HER2          | Phase III, First line | Lapa + let: 8.3 months |
|                      | Lapatinib      | n = 1286 | Let: 3 months |

Dasa, dasatinib; let, letrozole; exe, exemestane; enti, entinostat; gan, ganitumab; gef, gefitinib; ana, anastrozole; ful, fulvestrant; trast, trastuzumab; lapa, lapatinib; SRC, sarcoma; HDAC, histone deacetylase inhibitors; PFS, progression-free survival.
regulation of critical cell processes such as growth, proliferation, cytoskeleton organization, transcription, protein synthesis, ribosome biogenesis and autophagy\(^{17,18}\) (Figure 1).

Hyperactivation of the PI3K/Akt/mTOR pathway will induce tumor adaptation to anti-estrogenic therapy and can be defined by a mutation of PI3K (catalytic domain, or helical), AKT mutation, loss of PTEN function (deletion or loss of expression, epigenetics) or by the regulatory function of proteins TSC1/TSC2 (tuberous sclerosis complex) (deletion–mutation) (Figure 2).

**Randomized clinical trials with mTOR inhibitors**

Several clinical studies have been conducted in patients with ER + (endocrine receptor) HER2-MBC by combining anti-estrogenic [selective estrogen receptor modulators (SERM), selective estrogen down regulators (SERD)], antiaromatase inhibitors (AI) with agents targeting PI3K/Akt/mTOR such as PI3K inhibitors (panspecific or specific to the subunit 110 α or δ), AKT inhibitors, mTOR inhibitors or inhibitors of both mTOR and PI3K (dual inhibitor).

The **Horizon** study, a randomized phase III study, compared the combination letrozole 2.5 mg daily/temsirolimus 30 mg daily (5 days every 2 weeks) to letrozole/placebo as first-line therapy in 1112 AI-naïve patients with ER\(^+\) MBC. This study didn’t show any difference in terms of PFS between the two arms: median PFS 9 months; hazard ratio 0.90; 95% confidence interval (CI), 0.76 to 1.07; \(p = 0.25\).\(^{19}\)

**TAMRAD** was the first open-label, phase II study randomizing postmenopausal women with ER+/HER2−, AI-resistant MBC to tamoxifen 20 mg/day combined with everolimus 10 mg/day \((n = 54)\) or tamoxifen 20 mg/day alone \((n = 57)\). Randomization was stratified, based on primary and secondary
hormone resistance. Everolimus improved the clinical benefit rate (CBR) (the primary endpoint), and time to progression (TTP). In retrospective biomarker analyses, a correlation was observed between late effectors of mTORC1 activation and Akt-independent mTORC1 activation, and an inverse correlation was observed between the canonical PI3K/Akt/mTOR pathway and everolimus efficacy. These exploratory analyses need to be validated in a larger prospective study.

The BOLERO-2 study was the first phase III study demonstrating a benefit in terms of PFS with inhibition of one of the activated adaptive pathways mediating resistance to HT. This randomized, double-blind phase III trial evaluated everolimus (10 mg/day) with exemestane (25 mg/day) versus placebo with exemestane 25 mg/day in 764 postmenopausal women with ER+ advanced breast cancer with prior exposure to nonsteroidal AI. The median number of previous treatments was 3. 84% of the patients had previous sensitivity to endocrine therapy. With a median follow up of 18 months, the experimental arm significantly reduced the risk of relapse with a hazard ratio of 0.45 (95% CI 0.38–0.54, \( p < 0.0001 \)) and a PFS of 7.8 months \textit{versus} 3.2 months in the placebo arm. The benefit was consistent regardless of the presence of visceral metastases and hormone sensitivity. Despite clinically and statistically significant extension of PFS, this combination did not confer any overall survival benefit. Preserving quality of life is an essential component of palliative care in the advanced cancer setting; quality of life provides essential information on disease burden and guides treatment decisions. The most common grade 3 or 4 adverse events (AEs) were stomatitis (8% in the everolimus arm \textit{versus} 1% in the exemestane plus placebo group), anemia (6% \textit{versus} <1%), dyspnea (4% \textit{versus} 1%), hyperglycemia (4% \textit{versus} <1%), fatigue (4% \textit{versus} 1%), and pneumonitis (3% \textit{versus} 0%). A post hoc analysis confirmed the clinical benefit with no adverse impact on health-related quality of life.

What may explain the differences between Horizon, TAMRAD and BOLERO-2?

Unlike BOLERO 2 and TAMRAD, the Horizon study is a first-line setting MBC with only 40% of the patients having received previous adjuvant
endocrine therapy. In Horizon, patients didn’t receive any aromatase inhibitor as adjuvant therapy, and likely didn’t have any hyperactivation of the PI3K/Akt/mTOR. MTOR inhibitor might be less effective without an adaptive mechanism as hyperactivation of the PI3K/Akt/mTOR, induced by hormone-therapy resistance.

There is a real heterogeneity of responses to these drugs and patient outcomes, and biomarker analysis may help identify patients who will derive the greatest benefit from everolimus. In an exploratory analysis of BOLERO-2, the benefit of everolimus was maintained regardless of the presence or absence of an alteration in PIK3CA, FGFR1, CCND1 or their respective pathways. However, when the patients were assigned to the subgroups on the basis of mutations in PIK3CA exon 20 or 9, PFS benefit of everolimus appeared to be greater in those with exon 9 mutation [hazard ratio 0.26, 95% CI (0.12–0.54)] than in those with exon 20 mutation [hazard ratio 0.56, CI (0.31–1)]. Moreover, these data suggest that tumor with low chromosomal instability (CIN) might benefit from the addition of everolimus to exemestane, with a median PFS gain of 5.5 months for patients with CIN score below the 75th percentiles [hazard ratio 0.39, CI (0.28–0.54)].

Recently, recurrent mutations have been identified in the estrogen receptor. Chandarlapaty and colleagues evaluated blood samples from 541 of the 724 patients enrolled in BOLERO-2. They detected a D538G ESR1 mutation in samples from 114 (21.1%) patients, a Y537S ESR1 mutation in samples from 72 (13.3%) patients, and double mutations in samples from 30 patients. Median overall survival was 32.1 months for patients with neither a D538G nor Y537S ESR1 mutation, 26 months for those with only a D538G mutation, 20 months for those with only a Y537S mutation, and 15.2 months for those with double mutations. Exploratory analyses suggested that patients with centrally confirmed oestrogen receptor+/progesterone receptor+ tumors are more likely to benefit from the addition of pictilisib to fulvestrant (7.2 months versus 3.7 months, hazard ratio, 0.46; 95% CI, 0.27–0.78), even if the subgroup analyses were limited by the sample size. The absence of efficacy could be explained by the toxicity of pictilisib, with 22% of patients discontinuing experimental treatment, and the numerous dose reductions. Alternative strategies with specific inhibition of subunit α PI3K (alpelisib) or mutated PI3K (taselisib) are ongoing with a putative improved therapeutic index. Another explanation of this failure was put forward by Bachelot and colleagues. There are no direct correlations between PI3K AKT activation and late effectors of mTORC1 activation. The PI3K mutation in HER2-negative breast cancer is associated with low histological grade, high hormone sensitivity, good prognosis, and low efficacy.

Randomized clinical trials with PI3K inhibitors
One phase II trial and one phase III trial evaluated a PI3K inhibitor in ER+/HER2-MBC.

Recently, the FERGI trial assessed the addition of PI3K inhibition to HT in the second-line setting. In this phase II trial, investigators randomized pictilisib (GDC-0941), a potent oral inhibitor of multiple class I PI3K kinase isoforms in combination with fulvestrant (n = 89) versus fulvestrant with placebo (n = 79) in patients with MBC resistant to AI, with or without PIK3CA mutation. This study demonstrated a nonsignificant benefit in PFS in the pictilisib arm compared with the placebo arm (6.2 months versus 3.8 months; hazard ratio, 0.77; 95% CI, 0.50–1.19) in the overall population. This improvement was independent of PIK3CA mutation status in the tumor. Exploratory subgroup analyses suggested that patients with centrally confirmed oestrogen receptor+/progesterone receptor+ tumors are more likely to benefit from the addition of pictilisib to fulvestrant (7.2 months versus 3.8 months; hazard ratio, 0.46; 95% CI, 0.27–0.78), even if the subgroup analyses were limited by the sample size. The absence of efficacy could be explained by the toxicity of pictilisib, with 22% of patients discontinuing experimental treatment, and the numerous dose reductions. Alternative strategies with specific inhibition of subunit α PI3K (alpelisib) or mutated PI3K (taselisib) are ongoing with a putative improved therapeutic index. Another explanation of this failure was put forward by Bachelot and colleagues. There are no direct correlations between PI3K AKT activation and late effectors of mTORC1 activation. The PI3K mutation in HER2-negative breast cancer is associated with low histological grade, high hormone sensitivity, good prognosis, and low
mTORC1 activity. And high-grade tumors are associated with mTORC1 activation and few PI3KCA mutations, a potential target of PI3K inhibitor.31–33 The oncogenic action of PI3K mutation–PI3K inhibition combined with endocrine therapy might be a target only in a subgroup of patients with MBC with endocrine resistance.

**BELLE-2** is a prospective randomized phase III study in 1147 patients with ABC whose disease progressed on an AI. This study compared buparlisib [a specific oral inhibitor of the pan-class I phosphatidylinositol 3-kinase (PI3K)] with fulvestrant with fulvestrant and placebo. The study was stratified according to the presence of visceral metastasis and activation of the PI3K pathway (PI3K mutation, PTEN loss of function) in the primary tumor. The primary endpoint was PFS in the overall population and in patients with activation of PI3K. **PIK3CA** mutation status in blood samples was also analyzed in a subset of 587 patients. In the overall study population, PFS was 6.9 months in the combination therapy group versus 5.0 months in the control group (hazard ratio 0.78, 0.67–0.89). As in the FERGI study, patients with activation of the PI3K pathway in their primary tumor had no improvement in PFS with buparlisib added to fulvestrant (hazard ratio = 0.76; 0.60–0.97). However, this combination improved the median PFS in patients with a PI3K mutation detected by circulating DNA, with a 44% reduction in risk for progression (7 months versus 3.2 months; hazard ratio = 0.56; 0.39–0.80). The combination of fulvestrant and buparlisib was associated with serious side effects leading to discontinuation of therapy in 13.2%. The most common grade 3 and 4 AEs were liver dysfunction (ALT: 26 versus 1%, AST: 18 versus 3%), rash (8 versus 0%), hyperglycemia (15 versus 0.2%), and mood disorders (4.4% versus 0.4%).34

The discrepant results regarding the PIK3CA status and the benefit of PI3K inhibitors can probably be explained by the fact that the primary tumor is not suitable for measuring PI3K activation which should probably be analyzed in the metastatic lesions or in circulating DNA at the time of metastatic relapse. In a study of 104 patients, synchronous genetic heterogeneity and changing PI3K mutational status were demonstrated between primary and metastatic breast tumors.35 The analysis of PI3K mutation status on the circulating tumor DNA (ctDNA) is more feasible, less invasive and more reliable as shown by the unpublished results of the BELLE-2 study. However, prospective well conducted studies are still needed to confirm whether the presence of a PIK3CA mutation in circulating tumor DNA can predict response to this treatment.

**CDK 4/6 inhibition in association to AI in a first- or second-line setting?**

The mechanisms of resistance to HT often include upregulation of PI3K signaling pathways and cell cycle regulation. CDK4/6 phosphatase and inactivates (retinoblastoma) tumor suppressor proteins, leading to dissociation of E2F transcription factors and transcriptional regulation of genes for G1/S transition and cell cycle progression. Mitogenic signals or growth factor receptor signaling pathways converge on the cyclin D/CDK4 or CDK6 pathways, like ER and PI3K/Akt/mTOR. The activation leading to Rb phosphorylation is associated with resistance to endocrine therapy.3,36 Rb dysfunction is associated with luminal B-type breast cancer and is predictive of a poor response to endocrine therapies. CDK 4/6 inhibitors reverse the endocrine resistance in preclinical studies.37

**PALOMA-1**, a phase II trial randomized letrozole combined with palbociclib (a highly selective, orally administered CDK4/6 inhibitor) versus letrozole alone in postmenopausal patients with ER-positive MBC in the frontline setting. Palbociclib was administered at a dose of 125 mg/day for 3 weeks out of 4. In cohort 1 (n = 66), patients were enrolled on the basis of their ER+/Her2- biomarker status alone, whereas in cohort 2 (n = 99), they were also required to have cancers with amplification of cyclin D1 (CCND1), loss of p16 (INK4A or CDKN2A), or both. A third of the patients in each group had received previous endocrine therapy and half of these individuals had previously received AI. The final analysis showed a doubling of PFS in the overall population from 10.2 months with letrozole alone to 20.2 months with the letrozole–palbociclib combination [risk ratio (RR) of 0.488] and a decrease in risk of progression of 51% in favor of the combination (p = 0.0004). This improvement was observed in all subgroups, independent of cyclin D1 amplification or loss of p16. The number of death events was small at the time of publication, but was not statistically different between the palbociclib plus letrozole arm and the letrozole alone arm (37.5 versus 33.3; p = 0.42). The safety profile of palbociclib is favorable, including primarily hematological toxicity with
50% of patients presenting with Grade 3 or 4 neutropenia. However, no febrile neutropenia was observed. Approximately 40% of patients required a dose reduction or a recovery period. A pivotal phase III study, **PALOMA-2**, with the same design as the PALOMA-1 study was presented at ASCO 2016 and confirmed the safety and the clinical benefit of palbociclib with median PFS at 24.8 months (P+L) versus 14.5 months (PLB+L) [hazard ratio = 0.58 (0.46–0.72), p < 0.000001].

Despite immature overall survival (OS) data, the FDA has approved palbociclib for ABC patients who had not received prior systemic therapy for their advanced disease. However, fulvestrant could be a new option in a first-line setting. Fulvestrant is a selective estrogen-receptor degrader that targets the function of the hormone receptor currently used in second-line after AI. A randomized open label phase II trial, the **FIRST** trial, compared fulvestrant (500 mg) with anastrozole in the first-line setting and suggested that fulvestrant was superior in TTP.41

The extension phase III trial with the same design **FALCON** in 462 postmenopausal patients confirms statistically significant improvement in PFS with fulvestrant versus anastrozole [hazard ratio 0.797 (95% confidence interval 0.637, 0.999); p = 0.0486; median PFS, 16.6 versus 13.8 months, respectively]. However, a subgroup analysis showed an even greater impact on PFS in patients whose disease had not spread to the liver or lungs at baseline (22.3 versus 13.8 months).42

Quality of life was similar between the two arms and the most common AEs were arthralgia (joint pain) (16.7% versus 10.3%) and hot flushes (11.4% versus 10.3%) for fulvestrant and anastrozole, respectively. Fulvestrant could be a first-line option for patients requiring a low-toxicity approach such as older patients, or those with low-volume disease, or those for whom adherence to oral treatment is complicated. It should be noted that in the FALCON trial, none of the patients had prior HT, even for the treatment of early breast cancer.

**PALOMA-3** is a phase III trial randomizing fulvestrant-placebo versus fulvestrant-palbociclib among 521 patients with ER+/HER2-metastatic breast cancer, previously treated with hormone therapy. Premenopausal women could be included if they had received treatment with an LHRH agonist. The randomization was 2:1, with stratification based on the presence of visceral metastases, prior sensitivity on HT and menopausal status. With a median follow-up of 8.9 months, the median PFS was 9.5 months for the fulvestrant–palbociclib combination versus 4.6 months for fulvestrant alone (hazard ratio: 0.46, 95% CI 0.36–0.59, p < 0.0001). The OS follow-up is in progress. The safety profile was similar to that observed in the PALOMA-1 study, with neutropenia and maintained quality of life being common to both arms. Neither PI3KCA status in ctDNA nor the level of ER level expression predicts the response to palbociclib. These results confirm remarkable efficacy of this new therapeutic class, and standard therapy in the metastatic setting is changing. Other CDK4/6 inhibitors are under development such as ribociclib or abemaciclib.

Ribociclib has been evaluated in the **MONALEESA-2** trial. This phase III study randomized 668 postmenopausal women with ER+ HER2 ABC, who had not received any prior systemic treatment. Patients received ribociclib (600 mg/day, 3 weeks on/1 week off) and letrozole (2.5 mg/day, continuous), or letrozole plus placebo. In the ribociclib arm, there was a 44% improvement in PFS compared with the placebo arm (hazard ratio: 0.556, p = 0.00000329). Median PFS was 14.7 months in the placebo arm, but was not reached in the ribociclib arm at data cut off. AEs were similar to those when using palbociclib, with 59.3% of neutropenia.45

The **MONARCH 1** study is the only phase II study that evaluated the single-agent activity and safety of a CDK 4/6 inhibitor, abemaciclib, in patients with refractory metastatic breast cancer whose disease had progressed following multiple prior treatments, including chemotherapy. With a median of three lines of prior therapy for advanced disease and at the 8-month interim analysis, the confirmed odds risk ratio was 17.4%, the clinical benefit rate (complete response + partial response + stable disease ≥6 months) was 42.4%, and median PFS was 5.7 months. The treatment was well tolerated with only 6.8% discontinuations for AEs.46

**MONARCH 2**, a phase III trial, compared abemaciclib plus fulvestrant versus placebo with fulvestrant in women with ER+ HER2- ABC. Patients who had experienced disease progression on or within 12 months of receiving endocrine treatment in the neoadjuvant or adjuvant setting or while receiving first-line endocrine therapy for metastatic disease. The primary endpoint is PFS.
MONARCH 3 is a phase III trial of abemaciclib in combination with AI in patients with HR+, HER2- ABC.

Regardless the CDK 4/6 inhibitor, biomarkers are needed to optimize endocrine therapy and the levels of estrogen (ER) and progesterone receptor (PgR) could be one. Low proliferation with a low Ki67 level and high oestrogen and progesterone receptor expression (progesterone receptor) are probably predictive of response to endocrine therapy in neoadjuvant BC. Low PgR and higher Ki67 expression seem to be associated with poor prognosis and help to strengthen HT. Indeed, in the TEXT and SOFT trials, Regan and colleagues showed that the combination of exemestane + ovarian function suppression (OFS) is more beneficial than tamoxifen (tam) alone or tam + OFS in adjuvant endocrine therapy for women with poor prognostic features. In patients with newly metastatic disease or disease recurring after adjuvant tamoxifen, negative or low-level ER could be a predictive factor of response to gefitinib (EGFR inhibitor) and tamoxifen. No biomarkers have been found to predict the response of CDK4/6 inhibitors. In the PALOMA-2 trial, biomarker analyses on cell-cycle-related genes using immunohistochemistry for ER, Rb, p16, cyclin D1, and Ki-67 revealed no additional markers with sensitivity to palbociclib. In patients with newly metastatic disease or disease recurring after adjuvant tamoxifen, negative or low-level ER could be a predictive factor of response to gefitinib (EGFR inhibitor) and tamoxifen.

Given these remarkable results, acquired resistance to CDK4/6 inhibitors will be an emerging clinical challenge.

**Novel combinations**

The interaction between the PI3K/AKT/mTOR and cyclin D–CDK4/6–INK4–Rb pathways is thought to play a critical role in ER-driven breast cancer and preclinical ER+ breast cancer models. Hyperactivation of PI3K/Akt/mTOR might be an adaptive mechanism leading to endocrine therapy and CDK4/6 inhibitor resistance, and combination strategies are widely evaluated. The combination of ribociclib, a CDK4/6 inhibitor (LEE011; LEE), alpelisib, an alpha isofrom of class I PI3K inhibitor (BYL719; BYL), and letrozole (LET) has recently shown enhanced activity versus each agent alone. A phase Ib combination of LET, LEE, and BYL has shown an acceptable safety profile and demonstrates preliminary clinical activity in heavily pretreated patients with ER+/HER2- ABC. A total of 15 patients discontinued treatment: 7 (19%) due to progression of disease (PD) and 8 (22%) due to AEs. The most frequent study drug-related AEs (all grades >35%) were: nausea (all grades, 44%; G3/4, 6%), hyperglycemia (44%; 17%), neutropenia (42%; 22%), and fatigue (36%; 11%). Among 27 evaluable patients, 2 (7%) had a partial response (PR), 4 (15%) had unconfirmed partial response, 6 (22%) had stable disease (SD), 6 (22%) had non-CR (complete response), non-PD, and 5 (19%) had PD as best overall response. Inhibition of the three pathways provides sustained downregulation of Ki67, potentially preventing a feedback mechanism and hence delaying progression through therapy. Future randomized studies will compare LET + LEE or BYL with LET + LEE + BYL.

In summary, practice-changing trials have been successfully conducted combining HT with targeted therapies, disrupting the adaptive mechanisms of resistance to HT. This illustrated the successful concept of adding therapies. Fulvestrant as monotherapy is also an option, but recent trials have shown that PFS benefits can be achieved from it being combined with an inhibitor of the cyclin D–CDK4/6–INK4–Rb pathways. Fulvestrant, in combination with a PI3K inhibitor could be another option, but trials are still ongoing. Everolimus + exemestane is also an option in a second-line setting in postmenopausal women with ER+ cancer with prior exposure to nonsteroidal anti-inflammatories (letrozole or anastrozole), despite the absence of OS improvement (Figure 3). New and robust biomarkers are needed to define what is the best combination and the best sequence of treatment for hormone refractory MBC. To monitor the dynamics of tumor genomics over time, reliable and reproducible biomarkers for given patients are urgently needed. And ctDNA seems to be a useful one. There is a real interest in integrating liquid biopsies in prospective combination trials, but the sensibility and reproducibility of these tests are still being evaluated.
Resistance to endocrine therapy and adaptive mechanism in advanced breast cancer.

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