Overcoming endocrine resistance in hormone receptor–positive breast cancer

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ABSTRACT

Endocrine therapy, a major modality in the treatment of hormone receptor (HR)–positive breast cancer (BCA), has improved outcomes in metastatic and nonmetastatic disease. However, a limiting factor to the use of endocrine therapy in BCA is resistance resulting from the development of escape pathways that promote the survival of cancer cells despite estrogen receptor (ER)–targeted therapy. The resistance pathways involve extensive cross-talk between ER and receptor tyrosine kinase growth factors [epidermal growth factor receptor, human epidermal growth factor receptor 2 (HER2), and insulin-like growth factor 1 receptor] and their downstream signalling pathways—most notably PI3K/AKT/mTOR and MAPK. In some cases, resistance develops as a result of genetic or epigenetic alterations in various components of the signalling pathways, such as overexpression of HER2 and ER co-activators, aberrant expression of cell-cycle regulators, and PIK3CA mutations. By combining endocrine therapy with various molecularly targeted agents and signal transduction inhibitors, some success has been achieved in overcoming and modulating endocrine resistance in HR-positive BCA. Established strategies include selective ER downregulators, anti-HER2 agents, mTOR (mechanistic target of rapamycin) inhibitors, and inhibitors of cyclin-dependent kinases 4 and 6. Inhibitors of PI3Kα are not currently a treatment option for women with HR-positive BCA outside the context of clinical trial. Ongoing clinical trials are exploring more agents that could be combined with endocrine therapy, and biomarkers that would help to guide decision-making and maximize clinical efficacy. In this review article, we address current treatment strategies for endocrine resistance, and we highlight future therapeutic targets in the endocrine pathway of BCA.

Key Words Breast cancer, hormone-positive disease, endocrine resistance, targeted therapy, metastatic breast cancer

INTRODUCTION

Breast cancer (BCA) is a heterogeneous disease encompassing several biologic subtypes that have different clinical behaviours and responses to treatment. The most common subtypes are hormone receptor (HR)–positive [estrogen (ER) or progesterone (PR) receptor–positive, or both], which together constitute the luminal subtype and account for about 75% of all cases1,2. The first evidence for the estrogen-dependent nature of BCA was obtained more than 100 years ago by observation of the regression of BCA after oophorectomy3. With more advances in cancer treatment, several anti-hormonal approaches were developed, making endocrine therapy one of the earliest targeted treatments in BCA. Established targeted endocrine strategies in BCA include selective ER modulators such as tamoxifen; aromatase inhibitors (AI), which block conversion of androgens to estrogens in peripheral tissues; selective ER downregulators (for example, fulvestrant); and ovarian suppression or ablation, which prevents endogenous production of estrogen by the ovaries. Those endocrine treatments have provided clinical benefit and tumour regression with favourable toxicity profiles for patients with metastatic and nonmetastatic HR-positive disease4. In contrast, targeting PR has demonstrated less impressive clinical results, with increased toxicity and side effects5,6. Currently, PR is considered a marker for endocrine sensitivity, with higher PR expression suggesting better sensitivity to endocrine blockade.

Resistance to endocrine therapies is a major factor limiting the use of those agents in ER-positive BCA. Approximately 50% of patients with metastatic ER-positive disease achieve a complete or partial response or stabilization of their tumour with endocrine therapy; for the remaining
patients, the benefit is limited because of intrinsic or de novo resistance. The experience of the latter patients underpins the hypothesis that ER is not the only survival pathway for these tumours and that escape pathways could have already developed that drive cancer cell survival despite the targeting of ER with endocrine therapy.

Overcoming endocrine resistance has been a major focus of recent clinical research, and a number of clinical trials have combined endocrine therapy with signal transduction inhibitors and molecularly targeted agents with the aim of modulating and overcoming potential resistance pathways. In this review, we highlight the available data on endocrine resistance in bc and discuss the most recent updates from major clinical trials that have targeted various molecular and signalling pathways involved in the development of endocrine resistance.

**Definition of Endocrine Therapy Resistance**

No standardized definition for endocrine therapy resistance in ER-positive bc has been established. The complexity of endocrine resistance makes it difficult to clearly define the various types of resistance (intrinsic vs. acquired). Furthermore, the clinical data are limited; most of the information has come from preclinical studies, which have to be further investigated in well-designed studies. Generally, resistance can take either the de novo form (present before starting any treatment) or the acquired form (develops during therapy after an initial period of response).

These are the common clinical scenarios of endocrine resistance:

- **De novo** resistance of metastatic disease to all hormonal therapies, or recurrence soon after the start of adjuvant hormonal therapy, with no response to further endocrine therapy
- **De novo** resistance to some hormonal therapy, but sensitive to others
- Acquired resistance after initial response to endocrine therapy, followed by shorter periods of response to serial endocrine therapies until the cancer becomes refractory to all endocrine agents

Clinical observation of cancers that respond to endocrine therapy after progression on another agent supports the existence of agent-specific and class-specific types of endocrine resistance. In contrast to patients having bc that recurs or progresses shortly after cessation of endocrine therapy, bc in patients who experience a prolonged treatment-free interval (more than 12 months) might not be endocrine-resistant, and those patients might benefit from continued endocrine therapy. Patients with high-burden or rapidly progressing metastatic disease that is life-threatening should be treated with systemic chemotherapy to work toward faster control of their disease. Endocrine therapy could then be offered to those experiencing a clinical response to chemotherapy.

**Complexity of ER Signalling Pathways**

Estrogen has important effects on cellular processes, including cell proliferation and survival. Those actions are mediated through estrogen binding to its receptors (ERα and ERβ). Activation of ERα is responsible for most estrogen effects on normal breast tissue (making an essential contribution to mammary development) and on cancerous breast tissue (leading to hormone-dependent tumour growth). The ER signalling pathway (Figure 1) consists of a complex biologic network that involves several regulators and “cross-talk” between ER and membrane receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and insulin-like growth factor 1 receptor (IGF1R). Those signalling pathways regulate gene expression and control a variety of functions such as cell growth, proliferation, and survival.

Activation of ER has nuclear (genomic) and nongenomic functions. Classical ER signalling leads to genomic functions through estrogen ligand–receptor binding, which
leads to dimerization of Erα that complex with co-activators and co-repressors. The complexes then bind to specific DNA sites called estrogen response elements that in turn regulate expression of estrogen-responsive genes that are important in physiologic and pathologic processes, including breast tumour proliferation and progression. Estrogen receptor–ligand complexes can also bind to other transcription factors such as activation protein 1, specificity protein 1, and nuclear factor κB, functioning as a co-regulator to facilitate their binding to serum response elements, which in turn regulate the transcriptional activity of those factors and their responsive genes, triggering the co-activators into a higher state of activity. That non-classical Erα transcriptional regulation mechanism was shown to be augmented, even in the absence of estrogen, under the stimulation of growth factors in cancer cells that are resistant to endocrine therapy.

Activation of Erα can also occur through downstream events of receptor tyrosine kinases (EGFR, HER2, IGF1R)—sometimes called “ligand-independent Erα activation.” Bidirectional cross-talk between those pathways at multiple levels has been described. For instance, estrogen can increase the expression of ligands such as transforming growth factor α and insulin-like growth factor 1 (IGF1) that activate growth factor receptor pathways. On the other hand, Erα signalling can downregulate EGFR and HER2 while increasing the expression of IGF1R, thus promoting the EGFR–IGF1R axis, which in turn enhances the tyrosine kinase signalling and mediates resistance to antiestrogen through downstream signalling of the MAPK (mitogen-activated protein kinase) and PI3K (phosphoinositide 3-kinase) pathways. The expression of PAX2 (a transcription factor) leads to HER2 repression, while activation of AIB1 (SRC-3) upregulates HER2 transcription. Preclinical study suggested that the balance between PAX2 and AIB1 influences tamoxifen resistance through the regulation of HER2. Expression of Erα and PGR can be further downregulated by activation of the PI3K/AKT (protein kinase B)/mTOR (mechanistic target of rapamycin) and MAPK pathways through growth factor receptors, which might reduce cellular dependency on estrogen, thereby bypassing the therapeutic approach of lowering estrogen levels by using either selective Erα modulators or AIs.

The nongenomic function can be mediated through Erα activation, inducing assembly of protein complexes that activate signalling cascades ultimately leading to transcription factor activation independent of Erα binding to DNA. That function can rapidly regulate cellular processes by interaction with various signalling pathways such as the ER–PI3K–Srk–FAK complex that activates AKT and Erα–Src–PELP1 complexes, which in turn activate ERK. The activated AKT or ERK stimulates cellular growth and at the same time protects cancer cells from apoptosis by phosphorylating and inactivating several key apoptotic molecules; those molecules in turn promote Erα-independent tumour growth.

**FACTORS ASSOCIATED WITH ENDOCRINE RESISTANCE**

The mechanisms of endocrine resistance in HR-positive bca are complex and diverse; multiple molecules and pathways have been implicated. Much information has come from preclinical studies with tamoxifen. Many of the broad concepts that emerged from those studies will probably apply to resistance to other endocrine therapies, and more recent investigations have collected data about other alternative pathways that better correlate with specific types of resistance.

**ER and Co-regulators**

The primary mechanism for de novo resistance to endocrine therapy is lack of Erα expression. Another intrinsic mechanism was described in patients with inactive alleles of cytochrome P4502D6 (CYP2D6), which leads to impaired enzymatic activity for converting tamoxifen to its active metabolite, endoxifen, thus decreasing the response to tamoxifen therapy. A deficiency of the CYP2D6 enzyme is inherited as an autosomal recessive trait and presents in 7% of the white population and about 1% of the Asian population.

A small proportion of patients develop acquired resistance because of Erα expression loss (15%–20%) or Erα mutations (fewer than 1% in the primary tumour). On the other hand, loss of pgr expression occurs more frequently and has also been associated with increased growth factor signalling and endocrine resistance, making tumours more clinically aggressive and leading to worse patient outcomes. Additionally, HER2 status can change with disease progression (14% of patients). Those findings have led to the recommendation for rebiopsy on progression in relapsed and metastatic disease to assess for changes in hormonal and HER2 receptors, which can affect treatment choices. As previously noted, the increased activity of specificity protein 1 and the dysregulation and overexpression of Erα co-activators, most notably AIB1, have also been implicated in tamoxifen resistance.

The recent discovery of acquired mutations in the gene encoding for Erα, ESR1, has implicated those mutations in the mechanism of resistance to estrogen deprivation therapy such as AIs or oophorectomy in metastatic HR-positive bca. Advances in deep-sequencing technologies have shown the feasibility of detecting ESR1 mutations in liquid biopsies, which are thought to represent the most important metastatic tumour sites. Liquid biopsies can be obtained through noninvasive measurement of circulating tumour cells or cell-free DNA in peripheral blood. The most frequent ESR1 mutations, which occur in the ligand-binding domain of Erα, include alterations in the amino acids clustering between sequence numbers 534 and 538. Those activating mutations in the ligand-binding domain lead to strong constitutive activation of Erα, with subsequent tumour-cell proliferation in the presence or absence of estrogen.

The reported incidence of ESR1 mutations in metastatic breast sites ranges from 12% to 20%; it reaches up to 39% in women who have experienced progression while taking AIs, and it is less than 1% in primary breast tumours in treatment-naïve women. The presence of ESR1 mutations has been associated with poorer prognosis and limited response to AIs. Interestingly, patients with ESR1 mutations who develop resistance to AIs still respond to antiestrogen therapies using fulvestrant or tamoxifen, with fulvestrant
having been associated with greater tumor inhibition in preclinical studies. Recent data have also shown the effectiveness of combined treatments with mtor inhibitors (bolero-2) and inhibitors of cyclin-dependent kinases (cdks) 4 and 6 (paloma 3) in patients with or without esr1 mutations who have metastatic hr-positive bca progressing on prior ai treatment31,32.

Receptor Tyrosine Kinase (Growth Factor) Signalling Pathways

Ongoing endocrine therapy in bca can cause adaptive changes that result in activation of alternative signalling pathways such that cancer cells are no longer dependent on estrogen stimulation for survival and proliferation25. Those signalling pathways involve bidirectional cross-talk between growth factors, cellular kinases, and estrogen pathways at multiple levels, which can lead to endocrine resistance34. Pathway activation can also occur through other mechanisms such as overexpression of growth factors or their receptors (egfr, her2, and igf1r) or activation of down-stream signalling (ptenk/akt/mtor and ras/mek/mapk), or both13,33,34. In some cases, deregulation and activation of those signalling pathways occur as a result of genetic or epigenetic alterations, such as her2 gene amplification, activating mutations in the ptenk catalytic subunit or loss of expression of the ptex tumour suppressor of the ptex pathway28,35. Notably, activation of egfr and her2 signalling has been recognized as one of the factors most prominently contributing to endocrine resistance36,37. An important limiting factor for the activity of anti-egfr treatments is the lack of a biomarker, with exception of her2, which has been shown to be predictive for response to anti-her2 therapy in both hr-positive and hr-negative disease.

Cell-Cycle Regulators

Endocrine therapy in bca has both cytostatic and cytotoxic effects, as supported by clinical data demonstrating reduced cellular proliferation, induction of apoptosis, and reduction in growth rate as a result of cell-cycle arrest in the g1 phase38,39. Molecules that control the cell cycle include positive and negative regulators that have an important role in the estrogen effect to control cell-cycle progression from g1 to the s phase. Aberrant expression of those molecules has been associated with endocrine resistance13,40. Overexpression of the positive regulators myc and cyclins e1 and d1 can lead to endocrine resistance either by activating cdkks, which are important in the g1 phase, or by relieving the inhibitory effects of the negative regulators p21 and p27 on the cdkks41,42. In addition, decreased expression of the cdk inhibitor (p21 or p27) and inactivation of the retinoblastoma tumour suppressor are also associated with resistance to endocrine therapy7,43,44.

Clinical Trials with Strategies to Overcome Endocrine Resistance

Selective Er Degrader or Downregulator

Fulvestrant is a steroidal selective er downregulator that binds er, blocks its function, and increases er degradation. Unlike other endocrine therapies, fulvestrant is administered as an intramuscular injection in postmenopausal women with hr-positive bca. The first approved dose (250 mg every 28 days) was shown in two randomized phase iii studies (0020 and 0021) to be as effective as anastrozole, and joint analysis of the studies showed no difference in median time to progression (mttpp) between fulvestrant and anastrozole for postmenopausal women who had progressed on prior endocrine therapy (5.5 months vs. 4.1 months, p = 0.48)45,47. In the 0025 clinical trial, which compared fulvestrant (250 mg) with tamoxifen in the first-line setting in advanced bca, the primary endpoint of time to progression (ttpp) was not different between the two arms (mttpp for fulvestrant vs. tamoxifen: 6.8 months vs. 8.3 months; p = 0.088). The secondary endpoints, including clinical benefit rate, time to treatment failure, and overall survival (os), favoured of tamoxifen therapy. Unexpectedly, the study showed that fulvestrant did not meet the criteria for noninferiority to tamoxifen in the intention-to-treat population48.

Initial and subsequent data showed that, compared with a loading dose approach (500 mg on day 0, 250 mg on day 14 of month 1, and then 250 mg monthly), which reaches steady state within 28 days, fulvestrant at a 250 mg dose takes 3–6 months to achieve steady state49, which might allow for the use of higher doses to achieve quicker response and to limit the possibility of early relapses50. In the effect trial, loading-dose fulvestrant was compared with exemestane in women who had progressed while taking a nonsteroidal ai. The results showed similar efficacy, and the mttpp was 3.7 months in both groups50.

Three studies have investigated the combination of loading-dose fulvestrant and ais; the contrasting results in those trials were likely related to the patient groups and their prior treatments. In the sofia and fact trials, no additional benefit was seen for the combination compared with the single agent. The study population in the sofia trial consisted of patients with acquired resistance to nonsteroidal ais, and the result showed similar median progression-free survival (mfps) for fulvestrant alone and fulvestrant–anastrozole (4.8 months vs. 4.4 months, p = 0.98), and for fulvestrant alone and exemestane alone (4.8 months vs. 3.4 months, p = 0.56)51. In the fact study, more than two thirds of enrollees had received prior anti-estrogen therapy, and the mttpp was not different between loading-dose fulvestrant plus anastrozole and anastrozole alone (10.8 months vs. 10.2 months, p = 0.91)52. However, the swog 0226 study, whose enrollees included approximately 40% with de novo metastatic disease and 60% without prior adjuvant tamoxifen therapy, found superior outcomes with the fulvestrant–anastrozole combination compared with anastrozole alone or sequential anastrozole and fulvestrant [mfps: 15 months vs. 13.5 months, p = 0.007; median os (mos) 47.7 months vs. 41.3 months, p = 0.05]53.

More data have shown that er downregulation using fulvestrant is a dose-dependent process. Approximately 70% er downregulation is observed with a single 250 mg dose; nearly 100% can be achieved with high-dose (hd) fulvestrant (500 mg monthly), which might offer greater antitumour activity with superior efficacy54. The phase iii confirm trial compared hd fulvestrant (500 mg on days 0, 14, and 28, and then every 28 days) with the approved dose (250 mg every 28 days) in postmenopausal women
with recurrent or metastatic 
ca in whom prior endocrine therapy had failed. Compared with the lower 250 mg dose, 
fulvestrant was found to be associated with increased 
24.8 months vs. 13.8 months; hazard ratio: 0.58; 
value of 0.0092. The updated analysis of final os showed that, compared 
fulvestrant 250 mg, 
fulvestrant was associated with an improved 4.1-month difference in mos (26.4 months vs. 
22.3 months, \( p = 0.02 \))\(^{56} \). 

More recently, 
fulvestrant was compared with 
anastrozole in the 
FALCON trial, which included endocrine therapy–naive postmenopausal women with locally 
advanced or metastatic 
hr-positive 
ca, finding a significantly 
improved mpfs in favour of 
fulvestrant compared with 
anastrozole (16.6 months vs. 13.8 months; hazard ratio: 
0.797; \( p = 0.0486 \)). A significantly enhanced treatment effect was seen in the subgroup analysis of patients having 
non-visceral disease (defined as skeletal, lymph-node, 
or soft-tissue metastasis) compared with those having 
visceral disease (mpfs: 22.3 months vs. 13.8 months). In 
contrast, for patients with visceral disease, no difference 
was observed between the two treatment groups (mpfs: 
13.8 months vs. 15.9 months). A post hoc interaction test 
to assess for the consistency of treatment effects across 
the visceral and non-visceral subgroups resulted in a 
\( p \) value of 0.0092. The os data for the study have not yet 
been reported\(^{57} \). 

The current data support the use of 
fulvestrant as initial therapy in postmenopausal women with advanced 
ca who are 
hr-positive and who have low-burden disease 
and no prior exposure to endocrine therapy. They also provide 
support for the use of 
fulvestrant as a single agent 
or in combination with other agents such as nonsteroidal 
ai or 
4/6 inhibitors in those who have progressed while taking prior endocrine therapy (see the discussion of the 
PALOMA-3 trial in the next subsection). 

### CDK 4/6 Inhibitors

The 
4/6 inhibitors are small molecules that interact 
with the cell-cycle machinery and interfere with the G1-
to-S phase transition, thus leading to cell-cycle arrest and 
inhaling cancer-cell growth. The efficacy of combining 
4/6 inhibitor with the 
ai letrozole was demonstrated in the 
PALOMA-1, PALOMA-2, and MONALEESA-2 studies in 
postmenopausal women who had not previously received 
 systemic treatment for 
hr-positive advanced 
ca\(^{58-60} \). Significant improvements in mpfs were observed for 
ribociclib and letrozole compared with letrozole alone in 
PALOMA-1 (20.2 months vs. 10.2 months; hazard ratio: 0.488; \( p = 0.0004 \)) and 
PALOMA-2 (24.8 months vs. 14.5 months; hazard ratio: 0.58; 
\( p = 0.0001 \))\(^{58,59} \), and for 
ribociclib and letrozole compared with 
letrozole alone in MONALEESA-2 (at the preplanned interim analysis, the mpfs was not reached vs. 14.7 months 
respectively; hazard ratio: 0.56; \( p < 0.0001 \))\(^{60} \). Subset analyses of the 
PALOMA-2 study confirmed a consistent 
benefit of combined palbociclib–letrozole in all subgroups, 
including in patients with visceral and non-visceral disease 
and in patients who had and had not received prior endocrine 
therapy. Both studies showed that 
4/6 inhibitor combined with 
letrozole improves efficacy, but with some 
additional toxicities, including neutropenia, leucopenia, 
and fatigue, and with hepatotoxicity and QTc prolongation 
being identified with ribociclib. However, the side effects 
can be successfully managed with appropriate supportive 
care, monitoring, and dose reductions. 

The randomized phase 
PALOMA-3 study compared the combination of palbociclib and 
fulvestrant alone. That study demonstrated a doubling of 
mpfs in women who had progressed while taking an 
ai or 
within 1 month of completion of 
ai for advanced disease, 
or within 12 months of completing adjuvant hormonal 
therapy (mpfs: 9.2 months for palbociclib–fulvestrant 
vs. 3.8 months for fulvestrant alone; hazard ratio: 0.42; 
\( p < 0.001 \)). The side effects were consistent with the toxicity 
profiles reported in earlier studies. Notably, the study 
population included pre- and perimenopausal women, who 
also received the luteinizing hormone–releasing hormone 
agonist 
, and study benefits were consistent in all 
subgroups regardless of menopausal status. Data for os were immature at the time of the interim analysis\(^{61} \). 

A number of clinical trials continue to assess 
4/6 inhibitors as treatment for 
ca. The phase 
MONALEESA-3 trial is exploring 
ribociclib–fulvestrant for patients with 
advanced 
ca who are treatment-naive or who have progressed on only 1 prior line of endocrine therapy. The 
phase 
MONALEESA-7 trial is exploring first-line endocrine 
therapy with 
ribociclib in combination with 
tamoxifen or a nonsteroidal 
ai and 
goserelin for premenopausal women with 
advanced 
hr-positive 
ca. 
Abemaciclib is another 
4/6 inhibitor that has shown single-agent activity in 
patients who have been heavily pretreated for metastatic 
hr-positive 
ca\(^{62} \), and ongoing phase 
trials are exploring 
its efficacy in combination with 
fulvestrant (MONARCH-2) 
or with a nonsteroidal 
ai (MONARCH-3) for patients with 
metastatic hr-positive 
ca. 

### Inhibitors of Growth Factor Signalling Pathways

As previously described, the 
egfr/ 
her family has been implicated in endocrine resistance. Blockade of both the 
er and growth factor receptor signalling pathways has been investigated in many clinical trials, with the aim to reverse 
the resistance, restore endocrine sensitivity, and delay the 
need for chemotherapy. Results of clinical studies targeting 
egfr in 
ca, including a small-molecule tyrosine kinase 
inhibitor of 
egfr (gefitinib) and the dual tyrosine kinase 
inhibitor of 
egfr in 
ca or with a nonsteroidal 
ai or 
her2 (lapatinib), have shown limited benefit when combined with endocrine therapy\(^{63,64} \). On the other hand, adding 
her2-targeted therapies to endocrine therapy has been associated with better outcomes than those achieved with endocrine therapy alone in patients with 
hr-positive advanced 
ca with 
her2 overexpression. 

The combination of the monoclonal antibody to 
her2 (trastuzumab) and 
anastrozole, without chemotherapy, was explored in the 
TANDEM phase clinical trial in postmenopausal women with 
her2-positive, hr-positive metastatic 
ca. Compared with patients receiving 
anastrozole alone, those receiving combination treatment experienced 
better mpfs (4.8 months vs. 2.4 months; hazard ratio: 0.63; 
\( p = 0.0016 \)). A numeric but nonsignificant improvement in 
os was observed in the combination arm (mos: 28.5 months 
vs. 23.9 months; \( p = 0.325 \)); however, 70% of patients in the 
anastrozole-alone arm crossed over to receive 
trastuzumab after progression\(^{65} \).
With the evolution in the therapy for **HER2-positive** bca, treatments combining chemotherapy and anti-**HER2** agents have significantly improved survival outcomes and should be considered the initial therapy for all patients with advanced **HER2-positive** disease, including those with **HR-positive** bca. Patients who experience good control of their disease could be offered endocrine therapy in addition to maintenance anti-**HER2** therapy after chemotherapy.50

**Pertuzumab** is a humanized monoclonal antibody that binds to subdomain II in the extracellular domain of **HER2**, inhibiting **HER2/HER3** heterodimerization, which then blocks downstream signalling pathways of **HER2** (MAPK and PI3K). **Dual HER2 blockade** with trastuzumab–pertuzumab plus docetaxel was shown (in the **CLEOPATRA** trial) to be associated with significantly improved **PFS** and **OS** in metastatic **HER2-positive** bca.66 The potential benefit of adding **pertuzumab** to **AI** and trastuzumab (with or without induction taxane chemotherapy) for first-line treatment of postmenopausal women with **HER2-positive**, **HR-positive** advanced bca was investigated in the phase II **PERTAIN** clinical trial. The primary analysis in that study showed a significantly improved **PFS** (by 3 months) in the added pertuzumab arm compared with the trastuzumab and **AI** arm (18.9 months vs. 15.8 months; **hazard ratio**: 0.65; **p** = 0.007). Notably, more than 50% of the patients in both arms had received induction taxane chemotherapy (docetaxel or paclitaxel for 18–24 weeks) combined with anti-**HER2** therapy before starting **AI**. Results in most subgroups favoured the pertuzumab arm, including the groups of patients who did (**hazard ratio**: 0.75; 95% **confidence interval**: 0.50 to 1.13) and did not receive induction chemotherapy (**hazard ratio**: 0.55; 95% **confidence interval**: 0.34 to 0.88).67 The ongoing phase III **DETECT V/CHEVENO** study is comparing chemotherapy with endocrine therapy in combination with dual **HER2-targeted** therapy (trastuzumab and pertuzumab) in patients with **HER2-positive**, **HR-positive** metastatic bca.

### Inhibitors of the PI3K/AKT/mTOR Pathway

The **PI3K/AKT/mTOR** signalling pathway is critical in many cellular processes controlling cell growth, proliferation, survival, and metabolism. Aberrations in this intracellular pathway have been implicated in development of many cancers and resistance to cancer therapy.68 Multiple therapeutic strategies have been developed to target various components of the pathway in combination with endocrine therapy for the treatment of bca with endocrine therapy resistance.

**Rapamycin**—and its analogs temsirolimus, everolimus, and deforolimus—inhibit **mTOR** activation, which is often involved in cancer-cell resistance to treatment.69 The addition of everolimus to the steroidal **AI** ( exemestane) was evaluated in comparison with exemestane alone in the phase III **BOLERO-2** trial, which included postmenopausal women with **HR-positive**, **HER2-negative** advanced bca who had progressed on prior nonsteroidal **AI**. The primary endpoint, **PFS**, was significantly better in the combination treatment arm (**MPFS**: 6.9 months vs. 2.8 months; **hazard ratio**: 0.43; **p** < 0.001). However, the mos, which was the secondary endpoint and which was improved by 4.4 months with everolimus–exemestane, was not statistically significant (31 months vs. 26.6 months; **hazard ratio**: 0.89; **p** = 0.14). Notably, more than 85% of patients in both arms received post-study therapies.70,71

Furthermore, **tamoxifen–everolimus** was assessed in the phase II **Tamrad** study, in which postmenopausal women with **HR-positive**, **HER2-negative** advanced bca who had received prior treatment with **AI** were randomized to everolimus–tamoxifen or to tamoxifen alone. The clinical benefit rate at 6 months (primary endpoint) was significantly better in the combination arm than in the tamoxifen-alone arm (61% vs. 42%, **exploratory p** = 0.045). The **TPP** and **OS** were also improved in the everolimus–tamoxifen arm (**MPFS**: 8.6 months vs. 4.5 months with tamoxifen alone; **PFS** = 0.002; mos: not reached vs. 32.9 months; **hazard ratio**: 0.45; **p** = 0.007 (from the last data update in September 2011))72. Notably, the benefit of everolimus was greater for patients with secondary hormone resistance (defined as relapse >6 months after stopping adjuvant **AI** or response for 6 months or more to **AI** in the metastatic setting)72.

In the most recent phase II study (**Pecog 0102**), the addition of everolimus to **AI** fulvestrant, compared with fulvestrant–placebo, doubled the **PFS** in postmenopausal women with **HR-positive**, **HER2-negative** metastatic **BcA** resistant to **AI** therapy (10.4 months vs. 5.1 months; **hazard ratio**: 0.6; **p** = 0.02)73.

The foregoing studies—**Bolero-2**, **Tamrad**, and **Pecog 0102**—show that the combination of everolimus with endocrine therapy was associated with improved efficacy for the treatment of postmenopausal women with advanced **HR-positive** bca that had progressed during prior endocrine therapy. However, the combined treatment has been associated with increased toxicities, including fatigue, stomatitis, pneumonitis, anemia, and metabolic abnormalities such as hyperglycemia.70–73

**Another mTOR inhibitor**, temsirolimus, was evaluated in the **Horizon** trial. That phase III study compared temsirolimus–letrozole with letrozole–placebo as first-line treatment in **AI-naïve** postmenopausal women with **HR-positive** advanced bca. Adding temsirolimus to letrozole did not improve the primary endpoint (**MPFS**: 8.9 months vs. 9 months; **hazard ratio**: 0.90; **p** = 0.25), and the combination was associated with higher rates of grades 3 and 4 adverse events. The contrasting results of **Horizon** compared with **Bolero-2** and **Tamrad** are likely related to differences in the study population: where **Bolero-2** and **Tamrad** included patients who had progressed on prior **AI** treatment, **Horizon** enrolled **AI-naïve** patients for first-line treatment.74

A recent meta-analysis considered four phase II and III randomized trials that investigated **mTOR** inhibitors (everolimus, temsirolimus, and sirolimus) in combination with endocrine therapy, comparing those combinations with endocrine therapy alone in metastatic luminal (**HR-positive** bca). The pooled analyses (2,147 patients) showed improved outcomes, including **TPP** or **PFS**, **OS**, and overall response rate, for the combination therapy. Adverse events—and, in particular, those graded 3 and 4—were mostly increased with combination therapy (asthenia, fatigue, stomatitis, diarrhea, pneumonitis, rash, and dyspnea). The benefit of using **mTOR** inhibitors should therefore be balanced with the patient’s quality of life, which could be degraded by the side effects and increased toxicity of these treatments.75
Alterations in the PI3K/AKT pathway are frequently associated with resistance to endocrine therapy in BCA. Somatic mutations in the PI3K catalytic subunit p110α (PIK3CA) are the most common genetic alterations in that pathway. Many PI3K inhibitors—including pan-PI3K agents, isoform-specific agents, and dual PI3K/mTor agents—have been developed and are being tested in combination with endocrine therapy in various-phase clinical trials. The phase III BELLE-2 clinical trial evaluated the addition of the pan-PI3K inhibitor (buparlisib) with fulvestrant in postmenopausal women with HR-positive, HER2-negative advanced BCA who had progressed on prior AIs. In contrast to BELLE-3, BELLE-2 excluded patients who had already received mTor inhibitor therapies. The study met its primary endpoint, demonstrating a modest mPFS improvement for buparlisib–fulvestrant compared with fulvestrant alone (6.9 months vs. 5 months; hazard ratio: 0.78; p < 0.001). However, patients with tumours harbouring PIK3CA mutations detected in circulating tumour DNA achieved a clinically meaningful mPFS improvement with the combination, but responded poorly to fulvestrant monotherapy. The phase III BELLE-3 trial used the same combination of buparlisib and fulvestrant in AI-treated patients with advanced BCA who had progressed on or after mTor inhibitor–based treatment. The mPFS was improved with buparlisib–fulvestrant (3.9 months vs. 1.8 months with fulvestrant alone; hazard ratio: 0.67; p < 0.001). Exploratory subgroup analyses found that the benefit with combined treatment was confined to patients with PIK3CA-mutant tumours and those with visceral disease. Approximately 90% of enrolled patients had progressed on or after treatment with an mTor inhibitor before randomization. Both BELLE-2 and BELLE-3 observed higher rates of toxicity in the combination arm, including elevation of transaminases, mood disorders, and hyperglycemia, suggesting that a pan-PI3K inhibitor in this clinical setting might be too toxic. Data for os are still immature.

In contrast to buparlisib, alpelisib is an α-specific PI3K inhibitor that is being assessed in the phase III SOLAR-1 trial in combination with fulvestrant in patients with HR-positive, HER2-negative advanced BCA after disease progression on prior AI therapy. The patients are being assigned to two cohorts (PIK3CA-mutant vs. non-mutant status in tumour tissue) before randomization. Another ongoing phase III trial, SANDPIPER, is combining taselisib, another α-specific PI3K inhibitor, with fulvestrant in postmenopausal women with HR-positive, HER2-negative, PIK3CA-mutant advanced BCA who progressed on prior AIs. The results of those studies will determine efficacy and toxicity and whether only tumours harbouring PIK3CA mutations should be treated with these specific targeted compounds.

Histone Deacetylase Inhibitors

Histone deacetylase (Hdac) can interact with a variety of non-histones such as transcription factors and co-regulators, with varying functional effects. The repression of ER at a transcriptional level by HDAC is a potential mechanism of resistance. Preclinical and clinical data have shown that HDAC inhibitors lower the levels of ER suppression transcription, induce degradation of cyclin D1, and enhance the antiproliferative effects of endocrine therapy. Inhibitors of HDAC therefore potentially offer a way to modulate the effects of HDAC and thus reverse endocrine resistance.

The efficacy of adding a HDAC inhibitor (entinostat) to continued AI therapy in women whose BCA has progressed is therefore being tested, and results are awaited (see NCT00828854 at http://ClinicalTrials.gov). The benefit of combining another HDAC inhibitor (vorinostat) with an AI is also being tested in women with metastatic disease who have previously derived clinical benefit from endocrine therapy (NCT01720602).

SUMMARY

Combining endocrine therapy with various molecular targets and signal transduction inhibitors has led to successes in overcoming and modulating endocrine resistance in HR-positive BCA. Established strategies based on the available scientific data include HDAC fulvestrant as monotherapy in the first-line treatment of postmenopausal women with low-burden disease; HDAC fulvestrant as monotherapy or in combination with other agents such as nonsteroidal AIs or CDK 4/6 inhibitors (for example, palbociclib, ribociclib) in patients who have progressed on prior endocrine therapy; a combination of CDK 4/6 inhibitors (palbociclib, ribociclib) with letrozole or other endocrine therapy as first-line treatment in patients with higher-risk disease, such as visceral metastasis; a combination of maintenance anti-HER2 therapies with AI or other endocrine therapy; and a combination of mTOR inhibition (everolimus) with exemestane, tamoxifen, or fulvestrant in patients who have progressed on nonsteroidal AI. Inhibitors of PI3K are not currently approved outside the context of clinical trials. The results of the ongoing SANDPIPER and SOLAR-1 clinical trials are needed before PI3K inhibitors can be considered in treatment strategies. Finally, participation in clinical trials should always be encouraged to explore more options that can be combined with endocrine therapy to modulate endocrine resistance and to find biomarkers that can help in decision-making and in maximizing the chances of future success.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood Current Oncology’s policy on disclosing conflicts of interest, and we declare the following interests: CBM has received grants from Eli Lilly, AstraZeneca, Pfizer, and Novartis, and has received honoraria or consulting fees from Eli Lilly, AstraZeneca, Pfizer, and Novartis. AAF has no disclosures to make.

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