World Journal of Clinical Oncology

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DOI: 10.5306/wjco.v5.i5.990

World J Clin Oncol 2014 December 10; 5(5): 990-1001
ISSN 2218-4333 (online)

REVIEW

Overcoming endocrine resistance in metastatic breast cancer: Current evidence and future directions

Andrea Milani, Elena Geuna, Gloria Mittica, Giorgio Valabrega

Andrea Milani, Elena Geuna, Gloria Mittica, Giorgio Valabrega, Institute for Cancer Research and Treatment at Candiolo, University of Torino Medical School, FPO (Fondazione del Piemonte per l'Oncologia), 10060 Candiolo, Italy
Andrea Milani, Elena Geuna, Gloria Mittica, Giorgio Valabrega, Department of Oncology, University of Torino Medical School, 10123 Torino, Italy
Author contributions: Milani A and Geuna E contributed equally to this work; Milani A and Geuna E conceived and wrote the manuscript; Mittica G helped perform the literature search and contributed to writing, revising, and editing the manuscript; Valabrega G supervised the work and contributed to writing, revising, and editing the manuscript.
Correspondence to: Giorgio Valabrega, MD, Institute for Cancer Research and Treatment at Candiolo, University of Torino Medical School, FPO (Fondazione del Piemonte per l'Oncologia), SP 142, Km. 3.95, 10060 Candiolo, Italy. giorgio.valabrega@ircc.it
Telephone: +39-01-19933283 Fax: +39-01-19933299
Received: February 13, 2014 Revised: April 4, 2014
Accepted: July 18, 2014
Published online: December 10, 2014

Abstract

About 75% of all breast cancers are estrogen receptor (ER)-positive. They generally have a more favorable clinical behavior, prognosis, and pattern of recurrence, and endocrine therapy forms the backbone of treatment. Anti-estrogens (such as tamoxifen and fulvestrant) and aromatase inhibitors (such as anastrozole, letrozole, and exemestane) can effectively control the disease and induce tumor responses in a large proportion of patients. However, the majority of patients progress during endocrine therapy (acquired resistance) and a proportion of patients may fail to respond to initial therapy (de novo resistance). Endocrine resistance is therefore of clinical concern and there is great interest in strategies that delay or circumvent it. A deeper knowledge of the molecular mechanisms that drive endocrine resistance has recently led to development of new strategies that have the promise to effectively overcome it. Many resistance mechanisms have been described, and the crosstalk between ER and growth factor receptor signaling pathways seems to represent one of the most relevant. Compounds that are able to inhibit key elements of these pathways and restore endocrine sensitivity have been studied and more are currently under development. The aim of this review is to summarize the molecular pathophysiology of endocrine resistance in breast cancer and its impact on current clinical management.

Key words: Everolimus; Mammalian target of rapamycin; PI3K inhibitors; Estrogen receptor; Endocrine resistance

Core tip: Endocrine therapy forms the backbone of treatment for hormone receptor (HR)-positive metastatic breast cancer (MBC) patients. Unfortunately, resistance to endocrine agents develops in the majority of patients. A deeper knowledge of the molecular mechanisms that drive endocrine resistance has boosted the development of strategies designed to overcome resistance to endocrine therapies. In particular, co-targeting of receptor tyrosine kinase and intracellular signaling pathways (such as the PI3K-Akt-mTOR pathway) has emerged as a particularly promising strategy. We predict that the development of new drugs with a strong underlying biological rationale will quickly result in more personalized treatment of patients with HR-positive MBC and further improve outcomes.

Milani A, Geuna E, Mittica G, Valabrega G. Overcoming endocrine resistance in metastatic breast cancer: Current evidence and future directions. *World J Clin Oncol* 2014; 5(5): 990-1001 Available from: URL: http://www.wjgnet.com/2218-4333/full/v5/i5/990.htm DOI: http://dx.doi.org/10.5306/wjco.v5.i5.990

INTRODUCTION

Breast cancer is a leading cause of female death worldwide[1]. There has been a continuous decline in mortality over recent years as a direct result of improvements in early diagnosis and increased availability of more effective treatments[2,3]. However, despite these improvements, metastatic breast cancer (MBC) remains a largely incurable disease and new treatments need to prolong survival, relieve symptoms, and delay progression.

Approximately 75% of breast cancers express either or both the estrogen receptor (ER) and progesterone receptor (PgR)[4]. Hormone receptor (HR)-positive and negative disease differ in terms of clinical behavior, prognosis, patterns of recurrence, and aggressiveness. Patients with HR-positive disease are likely to have more indolent disease, bone metastases, and late recurrences[5]. For most HR-positive MBC patients, endocrine therapy is the preferential initial treatment and has a positive impact on survival.

Recently, a number of compounds with different mechanisms of action, low toxicity, and superior efficacy have become available for patients with HR-positive disease. Three classes of endocrine therapies are commonly used to treat HR-positive MBC: selective estrogen receptor modifiers (SERMs), such as tamoxifen, which directly bind to the ER and block its transcriptional activity; selective estrogen receptor downregulators (SERDs), such as fulvestrant, which bind to ER and induce its degradation; and aromatase inhibitors (AIs), such as letrozole, anastrozole, and exemestane, which reduce the production of estrogen *via* inhibition of the aromatase enzyme in peripheral tissues and within the tumor itself[6].

Unfortunately, although long-term remission is possible[7], the majority of patients develop resistance to endocrine therapy[8]. Moreover, a proportion of patients may have primary resistance to endocrine therapy[9]. There is therefore a lot of interest in developing strategies that delay the onset of endocrine resistance or circumvent acquired resistance to specific drugs.

It has recently been suggested that dysregulation of growth factor signaling networks and crosstalk between overexpressed growth factor receptors and ER play an important role in the endocrine-resistant phenotype[10]. Manipulating these networks is an attractive and potentially effective strategy that aims to delay the onset, or eventually overcome, resistance to endocrine therapies.

The aims of this review are to provide an overview of the known mechanisms of resistance to endocrine therapies and to focus on emerging strategies aimed at circumventing its development.

THE BIOLOGY OF THE ER

The ER is mainly a nuclear protein that modulates gene expression *via* several different pathways. A schematic of the biology of ER signaling is presented in Figure 1.

The "classical" pathway

Estrogen is a steroidal hormone that passively diffuses through cell membranes to enter the cell. The "classical" ER pathway is initiated by estrogen-induced dimerization of ER and subsequent binding to specific DNA promoter regions, known as estrogen response elements (EREs), which activates transcription of genes involved in promoting cellular proliferation and survival[11]. ER can also inhibit gene expression, particularly those involved in downregulation of the cell cycle or pro-apoptotic actions. The transcriptional activity of ER is regulated by a number of co-activators (for example, members of the p160 family of nuclear receptor co-activators such as SRC1 and SRC2) that bind to ER to form large complexes[12,13]. In breast cancer cells, SERMs such as tamoxifen lead to the formation of ER-co-repressor complexes that inhibit ER-dependent transcriptional activity to induce antiproliferative and pro-apoptotic effects.

The "non-classical" pathway

In addition to the "classical" regulation of gene expression, ER also regulates genes that do not harbor EREs in their promoter regions in a "non-classical" manner. ER can, in fact, interact with other proteins that are known to be involved in promoting gene expression, such as Fos and Jun[14].

Non-nuclear activities of the ER

Although the majority of cellular ER localizes in the nucleus, the ER can also localize in the cytoplasm and cell membrane, where it can interacts with receptor tyrosine kinase (RTK) growth factor receptors, such as the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2 (HER2), or insulin-like growth factor-1 receptor (IGF-1R)[15]. In fact, the ER plays a key role in this complex intracellular signaling network and is strictly linked to other signaling networks[16]. A complex network of bi-directional crosstalk exists at multiple levels in breast cancer cells, whereby the ER pathway and growth factor receptor signaling pathways interact and potentiate one another, resulting in dysregulated proliferation and growth[12].

Therefore, through direct DNA binding, co-activation, or molecular crosstalk, ER can influence tumor cell proliferation, survival, and malignant progression by amplifying the intracellular proliferative signals from RTKs and their downstream effectors.

Putative mechanisms of endocrine resistance

There is strong evidence that crosstalk between growth factor receptor and ER pathways can mediate resistance to endocrine therapy. The ER exists as part of a highly complex and adaptive signaling network that enables cancer cells to escape simple perturbations, such as those presented by the currently available endocrine therapies.

For example, overexpression of members of the EGFR family of RTKs, particularly HER2, has been described as a molecular alteration that is able to confer *de novo* resistance to anti-estrogens[12]. HER2 directly phosphorylates ER and its co-regulators, leading to enhanced

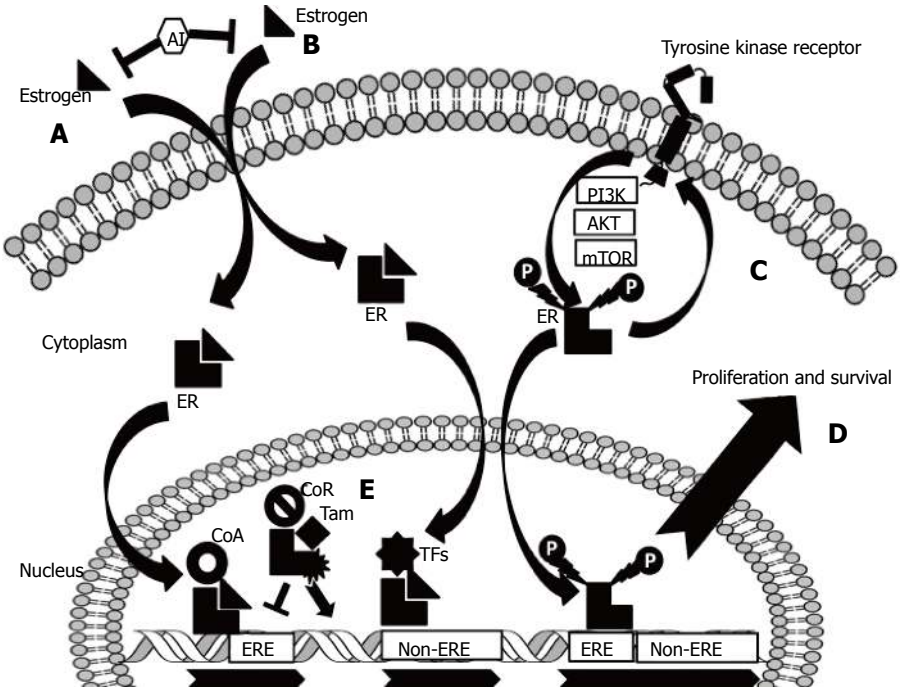

Figure 1 The biology of the estrogen receptor and a schematic representation of the key mechanisms of endocrine resistance. A: Estrogen induces gene regulation *via* the "classical" pathway. Estrogen passively diffuses through cell membranes and binds to the estrogen receptor (ER), inducing receptor dimerization. This complex recruits co-activators (CoA) and binds regions of DNA known as estrogen response elements (EREs), promoting transcription. Aromatase inhibitors (AIs) negatively regulate ER activity by reducing circulating estrogen levels; B: The ER can also cooperate with other transcription factors (TFs) and regulate the transcription of genes not harbouring EREs *via* the "non-classical" pathway; C: ER strictly interacts with receptor tyrosine kinases (RTKs) *via* their downstream effectors. ER can, in fact, be directly phosphorylated and activated, the final result being gene expression and a cascade of second intracellular effectors (the non-nuclear activity of ER); D: This strict and bi-directional crosstalk between ER and RTKs and downstream effectors is responsible for endocrine resistance; E: In breast cancer cells, SERMs [such as tamoxifen (Tam)] bind ER and induce the recruitment of co-repressors (CoR) that negatively regulate the activity of ER. Mutated forms of ER are able to enhance gene expression in spite of the presence of Tam.

ligand-independent gene expression, even in the presence of negative regulators such as SERMs.

There are data to suggest that patients with early breast cancers that overexpress HER2 obtain less benefit from adjuvant tamoxifen than those with HER2-negative tumors; furthermore, HER2 overexpression seems to be predictive of a poor clinical response to tamoxifen[17,18]. EGFR overexpression is also predictive of decreased benefit from tamoxifen[19,20] and increased risk of disease progression during anti-estrogen treatment[21].

There is emerging evidence to suggest that long-term estrogen deprivation can directly induce the transcription of growth factor receptors such as EGFR, HER2, and IGF-1R, resulting in increased activity of their downstream mediators and increased cellular proliferation, the final result being escape from estrogen deprivation and ligand-autonomous growth[22-24].

Another interaction that seems to be crucial in mediating resistance to endocrine therapies involves the phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway, an ubiquitous signal transduction pathway that is also interconnected with other RTKs, including, but not limited to, the EGFR family (Figure 1)[25-27]. This pathway regulates many cellular functions, not least growth and proliferation, differentiation, metabolism, migration, and survival[28], and it is abnormally activated in many different cancer types, including breast cancer, in which it has an important role in the development of anti-cancer drug resistance.

Dysregulation of this pathway is crucial in the development of acquired endocrine resistance. The pathway can become activated *via* increased upstream signaling due to activation of RTKs, PI3K-activating mutations, or decreased expression of negative regulators of the pathway, such as through loss of the tumor suppressor PTEN (phosphatase and tensin homolog). For example, several studies have established a link between upregulated Akt protein expression and/or phosphorylation and resistance to endocrine therapy[29,30], and it is known that an mTOR subunit phosphorylates and activates the functional domain 1 of the ER[31,32].

In a preclinical study, deGraffenried *et al*[33] reported that breast cancer cells with high Akt activity are resistant to hormonal therapy but that sensitivity could be restored with the use of mTOR inhibitors. Furthermore, in another study of ER-positive breast cancer cells, a combination of mTOR inhibitor and letrozole acted synergistically to inhibit proliferation and trigger apoptosis[34].

However, several other mechanisms have been described that contribute to endocrine resistance. For example, loss of ER expression in the evolution from primary to metastatic disease may contribute to the emergence of estrogen resistance; data from clinical studies suggest that 17% of ER-positive patients treated with adjuvant

tamoxifen may convert to an ER-negative phenotype at the time of relapse[35].

Mutations in *ESR1,* the gene encoding ER, also seem to negatively affect responses to hormonal therapy[36,37]. Recently Toy *et al*[36] reported frequent mutations in *ESR1* that affect the ligand-binding domain (LBD) of ER in metastatic hormone-resistant breast cancers after prolonged exposure to hormonal therapy. These highly recurrent mutations mainly affected p.Tyr537Ser, p.Tyr537Asn, and p.Asp538Gly, and as a consequence caused an agonist conformation of the receptor. In addition, they noted that LBD-mutant receptors have a hormone-independent active state that is likely to promote resistance to estrogen-depriving therapies. Interestingly, mutant ER retains some sensitivity to drugs that directly target the receptor, suggesting that more potent ER antagonists may be of substantial therapeutic benefit in this subgroup of individuals.

There may also be individual biological variability in drug metabolism that might influence responses to therapy. For example, about 8% of Caucasian women fail to convert tamoxifen to its active metabolite, endoxifen, which has been suggested to be a mechanism of *de novo* resistance[38].

In summary, multiple complex and adaptive mechanisms contribute to the development of endocrine resistance (Figure 1). As our understanding of the mechanisms that underpin resistance improves, the goal of future studies is to prolong responses to endocrine manipulation and potentially restore endocrine sensitivity in those tumors that have become resistant, with or without drugs that target interconnected pathways. Based on this theory, we describe three different approaches to overcome endocrine resistance that have recently been explored clinically in randomized trials.

OVERCOMING ENDOCRINE RESISTANCE

Combined inhibition of the ER and RTKs

Combined inhibition with ER- and HER2-targeting agents: HER2 is amplified and/or overexpressed (positive) in around 15% to 20% of human breast cancers. Although overexpression of HER2 is a marker of aggressiveness and poor prognosis, HER2-positive cells are sensitive to anti-HER2 targeted therapy, such as trastuzumab[39,40]. About half of HER2-positive breast cancers co-express hormone receptors and this is associated with resistance to both tamoxifen and AIs, as shown in a number of pre-clinical and clinical studies[25]. As a result of this pre-clinical evidence, several trials have explored using a combination of endocrine and HER2-targeting agents to overcome endocrine resistance.

Specifically, three trials have been published to date. The "Trastuzumab and Anastrozole Directed Against ER-Positive HER2-Positive Mammary Carcinoma" (TanDEM) phase 3 study compared anastrozole alone with the combination of anastrozole and trastuzumab as first-line treatment for patients with HER2/HR-positive advanced breast cancer[41]. The results showed that the combination of trastuzumab and anastrozole doubled median progression free survival (PFS) (2.4 mo *vs* 4.8 mo) and significantly increased the overall response rate (ORR) (6.8% *vs* 20.3%), compared to anastrozole alone. Side effects were modest and manageable (maximum grade 2) and consisted mainly of fatigue, vomiting, diarrhea, pyrexia, and arthralgia. There was no statistically significant treatment difference in overall survival; however, this may have been due to 70% of patients in the anastrozole arm crossing over to receive trastuzumab after progression on anastrozole alone.

The "Efficacy and Safety of Letrozole Combined With Trastuzumab in Patients With Metastatic Breast" (eLEcTRA) study prematurely closed due to slow recruitment. The design was the same as TanDEM but a different AI (letrozole) was prescribed[42]. Similar to TanDEM, eLEcTRA showed that the addition of trastuzumab to letrozole was associated with improved PFS and clinical benefit rate (CBR) at the cost of a modest increase in overall toxicity.

The third study was "EGF30008", a large, phase 3, double-blind, randomized-controlled trial conducted in 1286 women with HR-positive breast cancer; they were not selected on the basis of HER2 status (of the 1286 patients enrolled, 219 had HER2-positive tumors)[43,44]. These patients were randomized to daily oral treatment with letrozole (2.5 mg) plus the dual HER1-HER2 tyrosine kinase inhibitor lapatinib (1500 mg) *vs* letrozole (2.5 mg) plus placebo. In the ER-positive/HER2-positive population (n = 219), the addition of lapatinib to letrozole resulted in a significantly lower risk of disease progression than with letrozole alone. The PFS was 8.2 mo in the combined arm *vs* 3.0 mo in the placebo arm. The ORR (28% *vs* 15%) and CBR (48% *vs* 29%) were also significantly greater in lapatinib treated women. In contrast to the other two studies, the addition of lapatinib was accompanied by a significant increase in the grade 1 and 2 side effects commonly associated with dual tyrosine kinase inhibition, namely diarrhea (68%) and cutaneous rash (46%). The impact of lapatinib plus letrozole on OS has not been reported. However, based upon a clinically meaningful increase in PFS, the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved lapatinib in combination with an aromatase inhibitor in this setting.

As expected, the HER2-negative patients enrolled in EGF30008 derived no benefit in PFS from the addition of lapatinib to letrozole. Interestingly, however, in the sub-group of "tamoxifen-resistant" patients (*i.e.*, those relapsing during or within six months from the completion of adjuvant tamoxifen treatment), the improvement in PFS was similar to HER2-positive patients, suggesting that the disruption of crosstalk between the ER and RTK signaling pathways might restore sensitivity to anti-estrogens.

Recently, Finn *et al*[45] showed that weak ER expression is associated with worse outcomes for postmeno-

pausal women with advanced HR-positive disease when treated with letrozole alone compared to a combination with lapatinib. Their data suggest that the population of patients with low quantitative expression of ER within the HER2-negative population may be most likely to benefit from the addition of lapatinib to letrozole, at least in terms of PFS improvement. They hypothesize that this benefit could be related to the anti-EGFR effect of lapatinib.

In conclusion, these three trials suggest that the combination of an anti-HER2 agent and an AI has significant clinical benefit and improves PFS compared to endocrine therapy alone. No significant differences in overall survival (OS) were observed in any of the trials, possibly due to the influence of crossover and/or the number of lines of treatment received after progression. Interestingly, these three trials also confirm that HER2-positive patients have relative endocrine resistance; in fact, women receiving endocrine therapy alone had response rates ranging only from 7% to 15% and median time-to-progression (TTP) ranging from 2.4 to 3.3 mo.

These three trials provide proof-of-concept that HER2-associated endocrine resistance may be reverted by targeting HER2 and that combination therapy represents a therapeutic opportunity for patients with these particular clinicopathological features.

Combined inhibition with ER- and EGFR-targeting agents

As previously discussed, the crosstalk between the ER and EGFR has been reported to be mediate endocrine resistance. Therefore, combination strategies have been evaluated in the clinic[12,46].

Although the clinical and prognostic role of EGFR in breast cancer has yet to be fully characterized and is mainly restricted to "basal-like" tumors, a few randomized trials have explored the effect of combined ER and EGFR targeting in women with MBCs not selected on the basis of EGFR status[47].

NCT00229697 was a randomized phase Ⅱ trial that evaluated the addition of the pure EGFR inhibitor gefitinib to tamoxifen in patients with HR-positive advanced breast cancer[48]. Patients with newly metastatic disease, or who had recurred after adjuvant tamoxifen, during/after adjuvant AI, or after first-line AI, were randomized to receive tamoxifen plus placebo or tamoxifen plus gefitinib. A trend towards benefit from the combination therapy was seen in patients with tamoxifen-sensitive disease, with an increase in median PFS from 8.8 to 10.9 mo. In the AI-resistant population, no improvement in outcome was observed.

Another randomized phase Ⅱ trial (NCT00077025), presented by Cristofanilli *et al*[49], evaluated the efficacy and tolerability of anastrozole combined with gefitinib or anastrozole with placebo in tamoxifen-resistant women with HR-positive MBC[49]. Unfortunately, this study was closed prematurely due to slow accrual, but the data that were gathered showed that PFS was longer in patients receiving the combination therapy than for those patients receiving anastrozole plus placebo (14.7 mo *vs* 8.4 mo).

Both of these studies suggest that the observed benefit of EGFR inhibition can be explained by EGFR activation as a mechanism of adaptation to tamoxifen inhibition. It would be therefore interesting to explore this association in an EGFR overexpressing population, like in the neoadjuvant study published by Polychronis *et al*[50]. In this study, both the combination of anastrozole and gefitinib and, interestingly, gefitinib alone showed clinical activity. Although the ORR was similar in both arms, patients assigned to gefitinib and anastrozole had a greater decrease in tumor proliferation (as measured by Ki67 labeling), than those assigned gefitinib and placebo.

Combined inhibition of the ER and PI3K-Akt-mTOR signaling

Crosstalk between the ER signaling pathway and the PI3K-Akt-mTOR signaling pathway is thought to play a crucial role in the development of resistance to endocrine therapy (Figure 2). Specifically, PI3K-Akt-mTOR pathway upregulation is associated with ligand-independent activation of ER and an associated increase in expression of genes regulated by ER, albeit in the presence of anti-estrogens[30]. Moreover, several studies have shown that this effect can be reverted using mTOR inhibitors, such as everolimus or temsirolimus[33,34].

These data provide a strong rationale for combining agents that target this pathway and anti-estrogens in an attempt to restore endocrine sensitivity. Based on this, we present a series of clinical studies below that explore the efficacy of this approach.

Everolimus

Everolimus, the 40-O-(2-hydroxyethyl) derivative of sirolimus (a rapamycin analogue), is an oral mTOR inhibitor that binds with high affinity to its intracellular receptor FKBP12, a protein belonging to the immunophilin family. The everolimus-FKBP12 complex interacts with mTOR to inhibit downstream signaling[51,52].

In the phase Ⅱ trial "TAMRAD", Bachelot *et al*[53] evaluated the efficacy and safety of everolimus in combination with tamoxifen in 111 patients with MBC who had relapsed after first line treatment with AIs. Fifty-four patients were randomized to receive everolimus 10 mg/d and tamoxifen 20 mg/d, and the remainder received tamoxifen alone. Patients were stratified in two sub-groups: those who progressed during or within six months after the end of treatment with adjuvant AIs or progressed during the first six months of AIs with metastatic disease were defined having *ex novo* or primary resistance, whereas those who relapsed six or more months after completion of adjuvant AIs or after the first six months of therapy with AIs with metastatic disease were defined having acquired resistance.

The CBR was higher in patients treated with everolimus (61% *vs* 42%; $P = 0.045$) and TTP was longer in the combination arm (8.6 mo *vs* 4.5 mo; HR 0.54; 95%CI:

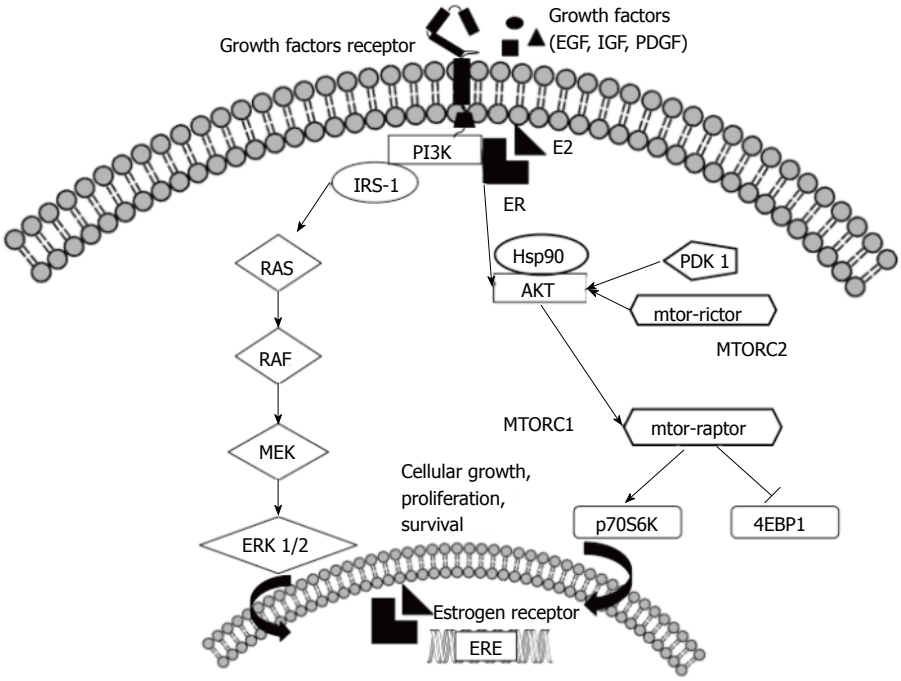

Figure 2 A representation of the molecular crosstalk between estrogen receptor and the receptor tyrosine kinases and PI3K-Akt-mTOR axes. In breast cancer, the PI3K-Akt-mTOR pathway modulates responses to signals communicated through growth factor receptors and the estrogen receptor (ER), and this crosstalk is important for sensitivity to anti-endocrine therapy. In particular, Akt and ERK1/2 phosphorylate ER on key residues involved in the induction of ligand-independent activation of DNA transcription. Furthermore, the converse occurs: estradiol, bound to membrane ER, interacts with and activates a regulatory subunit of PI3K. The mammalian target of rapamycin (mTOR) signaling cascade is another key regulatory pathway that controls proliferation and survival in cancer cells and plays an important role in the molecular crosstalk with the ER pathway. Two mTOR-interacting proteins, raptor and rictor, define distinct branches of the mTOR pathway: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Both active mTORC1 (*via* the phosphorylation of downstream targets, such as 4E-BP1 and p70S6 Kinase) and active mTORC2 contribute to promoting cellular survival and proliferation. EGF: Epidermal growth factor; IGF: Insulin-like growth factor; PDGF: Platelet derived growth factor; PI3K: Phosphatidylinositol-3-phosphate kinase; E2: Estradiol; IRS-1: Insulin receptor substrate-1; RAS–RAF–MEK–ERK: Mitogen activated protein kinase pathway; HSP90: Heat shock protein 90; PDK-1: Pyruvate dehydrogenase lipoamide kinase isozyme 1; p70S6K: Protein 70S6 kinase; 4EBP1: Eukaryotic translation initiation factor 4E-binding protein 1; ERE: Estrogen response element.

0.36-0.81). The subgroup analysis showed that the benefit of the combination therapy was greater in patients with acquired resistance (74% in the secondary resistance subgroup *vs* 46% in the primary resistance subgroup).

Baselga *et al*[54] explored the activity of this combination in the neoadjuvant setting. In a phase Ⅱ trial, 270 postmenopausal patients with ER-positive breast cancer were randomized to receive letrozole 2.5 mg/d plus everolimus 10 mg/d or letrozole 2.5 mg/d plus placebo for 16 wk prior to surgery. The primary endpoint was clinical response. The clinical response rates were 68.1% *vs* 59.1% in the combination and placebo arms, respectively ($P = 0.062$). Moreover, everolimus showed greater anti-proliferative activity (57% *vs* 30% in the everolimus and placebo arm, respectively; $P < 0.01$), defined as the reduction in cell proliferation assessed in pre- and post-surgical biopsy specimens.

Following these phase Ⅱ results, a larger randomized, double-blind, phase Ⅲ study was conducted by the same group[55]. The 'BOLERO-2' study enrolled 724 patients with HR-positive advanced breast cancer who had recurred or progressed after previous therapy with a non-steroidal AI (letrozole or anastrozole). Patients were randomized to receive exemestane 25 mg/d plus everolimus 10 mg/d or exemestane 25 mg/d plus placebo. The primary endpoint was PFS and the secondary endpoints were OS, ORR, CBR, safety, and quality of life.

The trial was stopped early because the pre-planned interim analysis showed a better PFS in the combination therapy arm (6.9 mo *vs* 2.8 mo in the combination and exemestane alone arms, respectively; $P < 0.001$) and a 57% reduction of risk of progression (HR = 0.43; 95%CI: 0.35-0.54; $P < 0.001$). These data were confirmed in the final PFS analysis conducted at a median follow-up of 18 mo[56]. PFS was 7.8 mo *vs* 3.2 mo (HR = 0.45; 95%CI: 0.38-0.54; $P < 0.001$) in the combination and placebo arms, respectively, and the magnitude of benefit was irrespective of clinicopathological characteristics, including previous treatment. The ORRs were 12.6% *vs* 1.7% ($P < 0.001$) in the combination and placebo arms, respectively; the CBR was better in the combination arm (51.3% *vs* 26.4% in the everolimus and placebo arms, respectively; $P < 0.001$). The final OS results are still not available and are awaited with interest.

Although generally well tolerated, all the clinical studies have reported toxicity related to everolimus. Data from BOLERO-2 showed that a greater proportion of patients discontinued treatment in the everolimus arm than in the placebo arm (19% *vs* 4%, respectively) due to adverse events. However, no significant difference

in overall quality of life was reported between the two arms[57]. The most commonly reported toxicities related to everolimus were stomatitis, fatigue, rash, anorexia, and diarrhea; a less common but life-threating adverse event was non-infectious pneumonia (presenting as an acute deterioration in respiratory function with ground glass or patchy opacities on computed tomography scans), which was reported in about 3% of patients. This non-infectious pneumonia seemed to be immunologically mediated and the clinical management often required immediate drug interruption and high doses of corticosteroids. Other concerning toxicities reported were hyperglycemia, hypercholesterolemia, and hypertriglyceridemia[58,59].

Temsirolimus

Temsirolimus is a compound that, similar to everolimus, inhibits the kinase activity of mTOR by complexing with FKBP12. However, it differs from everolimus in its pharmacokinetics and toxicity profile[60].

In a randomized phase Ⅱ study, Carpenter *et al*[61] explored the activity and safety of oral temsirolimus with letrozole in heavily pre-treated ER-positive MBC patients. This trial had a three-arm design: one arm received letrozole alone, whereas the other two arms received letrozole plus temsirolimus daily (10 mg) or intermittently (30 mg), respectively. One-year PFS was higher in both combination arms with letrozole alone (69%, 62%, and 48%, respectively).

However, these results were not confirmed in a subsequent larger randomized phase Ⅲ trial conducted by Chow *et al*[62] in heavily pre-treated MBC patients; no improvement in PFS was seen in the investigational arm and the study was stopped early.

Temsirolimus has also been evaluated in AI-naïve patients. In a randomized phase Ⅲ study, 1112 postmenopausal women with ER-positive locally advanced or metastatic BC with no prior exposure to AIs were randomly assigned to receive letrozole plus oral temsirolimus 30 mg/d for five days every two weeks or placebo with the same schedule[63]. The independent data monitoring committee also stopped this trial early at the second predefined interim analysis because the study was deemed unlikely to reach its primary endpoint. The published data showed no difference in PFS (8.9 and 9.0 mo, respectively; $P = 0.25$) between the groups at a median follow-up of 9.5 mo.

PI3K inhibitors

Alterations in the *PIK3CA* gene are the most common somatic mutations in breast cancer, and both crosstalk between the ER and PI3K pathways and PI3K activation are thought to play a role in endocrine resistance[64,65].

Specifically, PI3K pathway alterations occur in about 70% of breast cancers and include mutations and/or amplifications of the genes encoding the PI3K catalytic subunits, p110α (*PIK3CA*) and p110β (*PIK3CB*), the PI3K regulatory subunit p85α (*PIK3R1*), and the PI3K effectors *AKT1*, *AKT2*, and *PDK1*. The loss of lipid phosphatases, such as PTEN and INPP4B (inositol polyphosphate-4-phosphatase type II), can also activate the pathway[66-69].

In 2012, the Cancer Genome Atlas Network described that luminal ER+ tumors commonly harbor PI3K mutations, 49% in luminal A and 32% in luminal B[70]. Fu *et al*[71] have recently shown that activation of RTK signaling induces transcription of growth-related genes and causes decreases in ER levels and activity, leading to an inferior response to endocrine therapy. Co-targeting this pathway with ER and PI3K inhibitors therefore appears to be a promising therapeutic opportunity for patients with ER+ breast cancer. In support of this, Fu *et al*[71] found that the combination of tamoxifen with a dual PI3K/mTOR inhibitor (BEZ-235) additively reduces cell growth in different ER-positive HER2-negative breast cancer cell line models[71,72]. Furthermore, Sanchez *et al*[73] suggested in pre-clinical testing that fulvestrant may sensitize long-term estrogen deprived ER+ breast cancer cells to the therapeutic effects of PI3K inhibitors, with an associated synergistic increase in apoptosis.

At the most recent San Antonio Breast Cancer Conference, Juric *et al*[74] presented results from a phase 1b study of the PI3Kα inhibitor GDC-0032 in combination with fulvestrant in patients with ER+ advanced breast cancer. GDC-0032 was administered to 17 patients at a range of doses (six to nine mg/d) in combination with fulvestrant 500 mg every four weeks (with loading dose of 500 mg at day one, 14, and 28). The combination appeared to be well tolerated and had promising preliminary efficacy, with a final recommended dose of six mg per day. No dose limiting toxicities (DLTs) were observed and the main adverse events were gastrointestinal toxicities (anorexia, nausea, and diarrhea), metabolic toxicity (hyperglycemia), and rash. Metabolic partial responses were observed in eight out of 11 patients (73%), including those previously treated with fulvestrant[75].

At the same conference, another phase 1 trial reported on BKM120, a novel oral pan-PI3K inhibitor, in combination with fulvestrant in postmenopausal women with ER-positive MBC. Fulvestrant 500 mg IM was administered monthly on day one of each 28-d cycle (following the loading dose) and BKM120 was administered daily on day one to 28 of each cycle. 18 patients have been treated at three doses of BKM (80 and 100 mg/d continuously and 100 mg/d, five days on and two days off). Both BKM120 100 mg schedules (continuous or intermittent) with fulvestrant were tolerable without DLTs. Liver toxicity (assessed by ALT) has been reported with BKM120, especially with continuous dosing, and often requires dose reduction but not interruption. The results of this trial were promising, with over 50% clinical benefit, one partial response, and five prolonged disease stabilizations.

Phase Ⅲ studies of this combination have also been started in the same setting and preliminary information was reported at the 2012 American Society of Clinical Oncology (ASCO) annual meeting. For example, the BELLE (buparlisib breast cancer clinical evaluation) tri-

Table 1 Ongoing clinical trials of PI3K inhibitors in combination with endocrine therapy in hormone receptor-positive metastatic breast cancer

Treatment	Disease conditions	Trial status	Trial number
Phase Ⅰ			
BYL719 + letrozole	Postmenopausal women hormone receptor-positive stage Ⅳ breast cancer	Ongoing	NCT01791478
BKM120 + fulvestrant	Postmenopausal women estrogen receptor-positive stage Ⅳ breast cancer	Ongoing	NCT01339442
BKM120 or BEZ235 + letrozole	Postmenopausal women hormone receptor-positive stage Ⅳ breast cancer	Ongoing, not recruiting	NCT01248494
XL147 or XL765 + letrozole	Postmenopausal women hormone receptor-positive stage Ⅳ breast cancer	Completed	NCT01082068
Phase Ⅱ			
PF-04691502 + exemestane *vs* exemestane alone	Estrogen receptor-positive stage Ⅳ breast cancer	Withdrawn prior to enrolment	NCT01658176
PF-4691502 + letrozole *vs* letrozole alone	Postmenopausal women estrogen receptor-positive early (phase Ⅱ) and advanced (phase Ⅰb) breast cancer	Terminated	NCT01430585
GDC-0941 or GDC-0980/placebo + fulvestrant	Postmenopausal women estrogen receptor-positive, AI treated, stage ⅢB-Ⅳ breast cancer	Ongoing	NCT01437566
Phase Ⅲ			
BKM120/placebo + fulvestrant	Postmenopausal women hormone receptor-positive, AI treated, stage ⅢB-Ⅳ breast cancer progressed on or after mTOR inhibitor-based treatment	Ongoing	NCT01633060
BKM120/placebo + fulvestrant	Postmenopausal women hormone receptor-positive, stage ⅢB-Ⅳ breast cancer refractory to AIs	Ongoing	NCT01610284

als are investigating the safety and efficacy of buparlisib (BKM120) with fulvestrant.

BELLE2 is a phase Ⅲ of BKM120 plus fulvestrant in HR-positive HER2-negative advanced breast cancer that has progressed on or after AI therapy, while BELLE3 is a similar phase Ⅲ trial in patients with advanced breast cancer previously treated with AIs and refractory to endocrine and mTOR inhibitor combination therapy. The results from these trials will not be available for a few years (NCT01610284 and NCT01633060). BELLE4 is a phase II, randomized, double-blind and placebo-controlled study of BKM120 in combination with paclitaxel in patients with HER2-negative, locally advanced or metastatic breast cancer, with or without PI3K pathway activation. Other combination trials using different PI3K inhibitors are currently recruiting, for example BYL719 with letrozole or fulvestrant, and ongoing trials of PI3K inhibitors combined with endocrine agents are summarized in Table 1.

Multiple targeting of ER

Although the functional crosstalk between different molecular pathways and ER are thought to be the largest contributor to the development of endocrine resistance, many other mechanisms have also been described. For example, cells that express mutated ER circumvent inhibition by tamoxifen or long-term estrogen deprivation, as described above, and due to its peculiar mechanism of action, fulvestrant appears to be more active in these situations. Fulvestrant mediates the down-modulation and accelerated degradation of ER, thereby reducing its activity and it availability to other interacting molecules. Moreover, preclinical data suggest that fulvestrant retains and enhances its antitumor activity in the low estrogen environment, such as in the presence of AIs[76]. These data support a strong rationale to explore the activity of combining fulvestrant with AIs.

To this end, three large randomized trials have assessed this approach in postmenopausal women with ER-positive MBC[77-79]. Mehta *et al*[79] explored the activity of fulvestrant (500 mg loading dose, followed by 250 mg on days 14 and 28 and monthly thereafter) in combination with anastrozole compared to anastrozole alone (1 mg/d in both arms) in the first-line setting in women with MBC previously exposed to AIs and tamoxifen in the adjuvant setting. Overall, the study was positive in terms of its primary endpoint, with a small but statistically significant 1.5-mo increase in median PFS. However, the combination was only beneficial in the tamoxifen-naive population. No differences in ORR and CBR were observed in the two arms of the trial.

In the second study, conducted by Bergh *et al*[77], women with HR-positive MBC were randomized to receive the same two treatments as above in the first-line setting. Sensitivity to AIs was defined as either no prior exposure or administration of these drugs in the adjuvant setting and relapse occurring after one year from completion of adjuvant endocrine therapy. This trial failed to show differences between the study arms in the primary endpoint of TTP, or in ORR, CBR, and OS.

In the third study, recently published by Johnston *et al*[78], patients with MBC resistant to AIs were randomized to fulvestrant (dose and schedule as above) plus anastrozole (1 mg/d), fulvestrant plus placebo or anastrozole, or to exemestane 25 mg/d. Patients were eligible if they progressed while on AIs after a period of at least 12 mo for adjuvant therapy or six months for metastatic disease.

This study also reported no differences in terms of PFS, OS, ORR, and CBR between the treatment arms.

CONCLUSIONS AND FUTURE PERSPECTIVES

Endocrine therapy was traditionally thought to be less effective than chemotherapy for the treatment of women with MBC and was consequently demoted to a secondary role. Recently, our understanding of ER biology has improved and, in parallel, our therapeutic armamentarium has expanded with the development of several classes of compounds with different mechanisms of action. As a result, endocrine therapy is the confirmed leader in the treatment of HR-positive MBC due to greater efficacy and negligible toxicity.

However, most women treated with endocrine therapies develop resistance, and several mechanisms of resistance have been described. In particular, ER appears to be a key player in a complex network of signaling pathways that leads to proliferation and survival of cancer cells. Due to the adaptability of this network, cells can easily escape simple perturbations, such as those presented by the currently available endocrine therapies.

Moreover, these observations have provided the rationale for developing drugs that target other interconnected pathways. Combinations of endocrine agents with or without these drugs have recently been tested in randomized trials, with exciting results.

In this paper, we have described three possible strategies to overcome endocrine resistance, some of which are already becoming part of clinical practice.

Of these, co-targeting the RTK signaling pathways and intracellular signaling networks is the most effective. Lapatinib has recently been approved in patients with HER2- and ER-positive breast cancer, and everolimus has been approved in combination with exemestane for women refractory to AIs. Many other drugs that target intracellular signaling networks, especially the PI3K-mTOR-Akt axis, are currently under development and some of these have shown promising results.

However, recent advances in the understanding of the biology of ER signaling and of the molecular markers of resistance have highlighted that ER and its pathway remain central to endocrine resistance. These findings are likely to translate into new strategies to overcome endocrine resistance in the near future. For example, targeting tumors with specific ER mutations with more potent and specific anti-estrogens seems to be a fascinating approach.

All these advances have positively impacted on survival of women with HR-positive MBC. They chart a course towards the biology-based selection of treatments and a more rational use of chemotherapy to improve efficacy and limit toxicity in women with breast cancer.

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