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Review

Everolimus in hormone receptor–positive advanced breast cancer: Targeting receptor-based mechanisms of resistance

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ABSTRACT

Although patients with hormone receptor (HR)–positive breast cancer are successfully treated with endocrine therapy, many tumors go on to develop resistance to these agents. Studies have determined that mechanisms of resistance to endocrine therapy are quite complex and can involve a multitude of signal transduction pathways, either through direct association with the estrogen receptor or through cross-talk with other pathways. Preclinical studies have suggested the therapeutic importance of the mammalian target of rapamycin (mTOR) pathway and that inhibiting this pathway may restore sensitivity to endocrine therapy. The oral mTOR inhibitor everolimus has been extensively studied for breast cancer. Clinical studies suggest that everolimus in combination with endocrine therapy improves progression-free survival and is well tolerated. A combined approach, targeting both mTOR signal transduction and the HR pathways, promises to take clinical research in a new direction for the treatment of HR-positive advanced breast cancer.

Introduction

Overview of breast cancer

Breast cancer is the most common malignancy in women. It is estimated that approximately 226,870 women will be diagnosed with and 39,510 women will die of breast cancer in the United

involve estrogen receptor (ER)/progesterone receptor (PR) status, human epidermal growth factor receptor-2 (HER2)/neu amplification status, grade, and stage of the disease.[2–4]

Endocrine therapy

Estrogen hormones play a major role in the development and progression of hormone receptor (HR)–positive breast cancer. The majority of breast tumors express ER and/or PR, and endocrine therapy has proven beneficial in all stages of the disease.[5,6] Multiple treatment strategies have been developed for the treatment of HR-positive breast cancer, including bilateral oophorectomy, ovarian suppression, and endocrine therapy.[7,8] More specifically, the selective ER modulator tamoxifen has improved survival both in early breast cancer and in metastatic disease. Tamoxifen has also decreased the incidence of breast cancer in at-risk patients and in women with ductal carcinoma in situ.[6,9] More recent therapeutic options include agents that either decrease estrogen production such as aromatase inhibitors[10] or degrade the receptor itself (i.e., fulvestrant).[11] These agents inhibit ER signaling as effectively as or

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have improved

thereby delaying the need for chemotherapy.

Endocrine therapy resistance

Despite the benefits of endocrine therapy, many ER/PR-expressing tumors do not respond to endocrine manipulation (de novo [or primary] resistance), and in some cases initially responsive tumors eventually progress (acquired [or secondary] resistance). Acquired resistance may occur while receiving therapy but also following completion of adjuvant endocrine therapy. Recent advances in the understanding of ER biology revealed an increasingly complex process of ER signaling and interaction with other growth factor signaling pathways in the cancer cell.[13] The mechanisms contributing to resistance to endocrine therapy are inherently complex. Following prolonged periods of endocrine therapy, resistance has been implicated through various growth factors and

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kinase pathways, including insulin-like growth factor receptor (IGFR), HER2, mitogen-activated protein kinases (MAPK), Src, epidermal growth factor receptor (EGFR), and mammalian target of rapamycin (mTOR).[14,15] The recognition of this molecular cross-talk between ER and other growth factor signaling pathways improves our understanding of the causes of endocrine resistance and identifies the potential for development of new therapeutic strategies to overcome endocrine resistance in breast cancer. These recent advances in understanding the complex interaction of ER signaling with other growth factor signaling pathways suggest that targeting the ER as an isolated pathway may not be an optimal therapeutic strategy. Rather, simultaneous inhibition of other active signaling elements that interact with ER may be necessary to improve endocrine response and prevent resistance. Exploring new targeted therapies in combination with classic endocrine modalities represents a new strategy in overcoming endocrine therapy resistance. Various inhibitors have been investigated for these targets, including agents directed at tyrosine kinase moieties, antibodies against surface growth factor receptors, or other drugs that target key signal transduction mediators of growth factor signaling, such as farnesyl transferase inhibitors and mTOR inhibitors.[16] To date, mTOR inhibitors appear to be the most promising.

Endocrine resistance: targeting mTOR

mTOR is a serine/threonine protein kinase that regulates cell growth, proliferation, motility, cell survival and apoptosis, protein synthesis, and metabolism (Fig. 1).[17,18] mTOR belongs to the phosphatidylinositol 3-kinase (PI3K)-related kinase protein family and integrates signaling from upstream pathways, including insulin, growth factors (such as IGF-1), and amino acids.[17] The protein exists as two distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[19]

mTOR is a downstream mediator in the PI3K/Akt-signaling pathway and is critical for cell survival.[20] Rapamycin and rapamycin analogs are specific mTOR antagonists that target this pathway and block the downstream signaling elements, resulting in cell cycle arrest in the G_1 phase.[21] In breast cancer, the PI3K/Akt pathway can be activated via the HER (or ErbB) family of growth factor receptors, the IGFR, and the ER.[22] This pathway has been demonstrated to play a central role in the development of resistance to trastuzumab and tamoxifen therapy.[23–25] Therefore, targeting the PI3K/Akt pathway with mTOR antagonists may increase their therapeutic efficacy.

Upstream regulators of the PI3K/Akt pathway include the tumor suppressor gene phosphatase and tensin homolog deleted from chromosome 10 (PTEN). PTEN inhibits the activity of PI3K, and the hyperactivation of PI3K/Akt signaling elements in PTEN-deficient malignancies suggests that these cancers are dependent on this pathway for growth and maintenance.[26] In fact, loss of PTEN suppressor gene function has been associated with Akt activation[20] and development of resistance to trastuzumab.[24]

Tumor cell lines lacking PTEN exhibited high sensitivity to the mTOR inhibitor temsirolimus.[27] Most breast cancer cell lines highly sensitive to temsirolimus were found to be estrogen dependent, to overexpress Erb-2, and/or to have PTEN deletions. Breast cancer cell lines resistant to temsirolimus treatment lacked these features. Breast cancer cell lines sensitive to the inhibitory effects of temsirolimus generally had higher levels of activated Akt, perhaps leading to downstream activation of mTOR and subsequent sensitivity to mTOR inhibitors.[28] Alternately, a mechanistic study of everolimus has shown PIK3CA mutations are selectively sensitive to mTOR inhibition while tumor cells with PTEN loss of function are not susceptible to pharmacological inhibition, suggesting activation of these two pathways have functionally distinct consequences.[29]

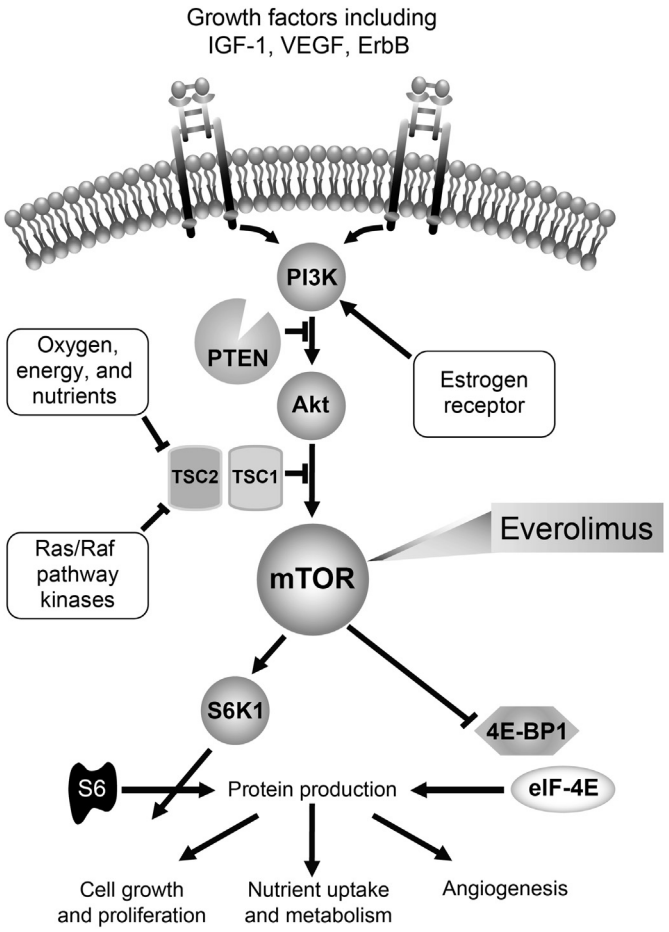

Fig. 1. Signaling pathways involved in interaction with mTOR. Akt, protein kinase B; 4E-BP1, 4E-binding protein 1; ErbB, epidermal growth factor; eIF-4E, eukaryotic initiation factor 4E; S6 = ribosomal protein S6; IGF-1, insulin-like growth factor-1; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted from chromosome 10; S6K1, S6 kinase 1; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.

Recently, both control MCF-7 cells and MCF-7/Aro (aromatase-expressing) cells were found to be susceptible to treatment with the rapamycin analog everolimus in vitro. Everolimus was found to almost completely inhibit estradiol-induced proliferation in control MCF-7 cells and estradiol and androstenedione-induced proliferation in MCF-7/Aro cells, suggesting that mTOR signaling is required for estrogen-regulated proliferative response in MCF-7 cells. Also, combination therapy with letrozole and everolimus resulted in increased inhibition of cell proliferation, and the two therapies demonstrated a synergistic effect.[30]

Evidence supporting the rationale for everolimus use in HR-positive breast cancer

Preclinical studies have shown that breast cancer cells with upregulated Akt signaling are resistant to hormonal therapy, but responsiveness to endocrine treatment may be restored by everolimus or other mTOR inhibitors.[31,32] In models of ER-positive breast cancer, small concentrations of everolimus reduced cell growth in vitro and increased antitumor activity of letrozole.[33]

An initial evaluation of temsirolimus with letrozole suggested the potential utility of mTOR inhibitors in combination with endocrine therapies as an effective treatment option in HR-positive breast cancer.[22,34] A phase III study of temsirolimus in combination with letrozole was subsequently conducted in patients with HR-

positive advanced breast cancer, regardless of whether or not they were previously treated with chemotherapy or hormonal therapy. However, the study showed no benefit of the temsirolimus/letrozole combination over letrozole alone in progression-free survival (PFS) (8.9 vs 9.0 months; hazard ratio = 0.90; 95% confidence interval [CI]; 0.76–1.07; $P = 0.250$).[35] Conversely, early-phase clinical trials suggested that everolimus may be a viable treatment option for HR-positive breast cancer. A phase I dose-escalating study of everolimus plus letrozole in postmenopausal women or men with stable metastatic breast cancer or progression after ≥4 months of first- or second-line therapy with letrozole alone ($n = 18$) found that one patient had a complete response lasting >22 months and another experienced a 28% reduction in liver metastases.[36] Subsequent clinical trials addressed the benefits of everolimus in combination with endocrine therapy (Table 1).[37–42] A phase II, randomized study of neoadjuvant everolimus plus letrozole versus placebo plus letrozole in patients with ER-positive breast cancer showed higher response rates in the everolimus arm compared with placebo (68.1% vs 59.1%, respectively; $P = 0.062$). Notable reductions in progesterone receptor and cyclin D1 expression were documented in both arms, whereas down-regulation of phosphorylated S6 protein occurred only in the everolimus arm. Significant reduction in Ki67 expression, a biomarker of cell proliferation, occurred in 57% of patients in the everolimus arm versus 30% of patients in the placebo arm ($P < 0.01$). Therefore, everolimus increased the efficacy of letrozole in the treatment of ER-positive breast cancer in a neoadjuvant setting in terms of both clinical response and antiproliferative effect.[41] Additionally, an ongoing phase II study of everolimus in combination with fulvestrant in patients ($n = 11$) with HR-positive metastatic breast cancer with aromatase inhibitor therapy failure within 6 months found that at the time of data presentation the median time to progression (TTP) was 8.6 months and the clinical benefit rate (CBR) was 55%.[42]

Table 1
Clinical trials of everolimus in HR-positive advanced breast cancer.

Study	Study design	Patients (n)	Treatments	Objective response rates/clinical benefit rate	Disease progression
Baselga et al., 2012[a,37]	Placebo-controlled, randomized phase III	Postmenopausal ER+ advanced BC ($n = 724$)	Exemestane (25 mg/day) + everolimus (10 mg/day)	ORR = 9.5% SD = 70.1%	PFS = 6.9 months
BOLERO-2			Exemestane (25 mg/day) + placebo	ORR = 0.4% ($P < 0.001$, vs placebo + exemestane) SD = 58.6%	PFS = 2.8 months (hazard ratio = 0.43; 95% CI: 0.35–0.54; $P < 0.001$)
Hortobagyi et al., 2011[a,38]	Placebo-controlled, randomized phase III	Postmenopausal ER+ advanced BC ($n = 724$)	Exemestane (25 mg/day) + everolimus (10 mg/day)	ORR = 12.0% CBR = 50.5%	PFS = 7.4 months
BOLERO-2 (12-month data update)			Exemestane (25 mg/day) + placebo	ORR = 1.3% ($P < 0.001$, vs placebo + exemestane) CBR = 25.5% ($P < 0.001$, vs placebo + exemestane)	PFS = 3.2 months (hazard ratio = 0.44; 95% CI: 0.36–0.53; $P < 0.001$)
Piccart et al., 2012[a,39]	Placebo-controlled, randomized phase III	Postmenopausal ER+ advanced BC ($n = 724$)	Exemestane (25 mg/day) + everolimus (10 mg/day)	ORR = 12.6% CBR = 51.3%	PFS = 7.8 months
BOLERO-2 (18-month data update)			Exemestane (25 mg/day) + placebo	ORR = 1.7% ($P < 0.001$, vs placebo + exemestane) CBR = 26.4% ($P < 0.001$, vs placebo + exemestane)	PFS = 3.2 months (hazard ratio = 0.45; 95% CI: 0.38–0.54; $P < 0.001$)
Bachelot et al., 2012[40]	Randomized phase II, Simon 2-stage	HR+, HER2− MBC	Tamoxifen (20 mg/day) + everolimus (10 mg/day) ($n = 54$)	CBR = 61%	TTP = 8.6 months
TAMRAD			Tamoxifen (20 mg/day) ($n = 57$)	CBR = 42% (exploratory $P = 0.045$ vs tamoxifen alone)	TTP = 4.5 months (exploratory $P = 0.002$)
Baselga et al., 2009[41]	Phase II randomized	Postmenopausal women with operable ER+ BC ($n = 270$)	Everolimus (10 mg/day) + neoadjuvant letrozole (2.5 mg/day) ($n = 138$)	ORR[b] = 68.1% (95% CI: 60.3–75.9)	Not evaluated
			Neoadjuvant letrozole (2.5 mg/day) + placebo ($n = 132$)	ORR[b] = 59.1% (95% CI: 50.7–67.5; $P = 0.062$, vs letrozole + placebo)	Not evaluated
Badin et al., 2010[42]	Phase II open-label	ER+ MBC with AI therapy failure within 6 months ($n = 11$)	Everolimus (10 mg/day) + fulvestrant (500 mg day 1, 250 mg day 14, 250 mg day 28, and monthly thereafter)	CBR = 55%	TTP = 8.6 months

AI, aromatase inhibitor; BC = breast cancer; CBR = clinical benefit rate (CR + PR + SD at 6 months); CI = confidence interval; CR = complete response; ER+, estrogen receptor–positive; HER2−, human epidural growth factor 2 receptor–negative; HR+, hormone receptor–positive; MBC = metastatic breast cancer; ORR = objective response rate; PR = partial response; pts = patients; SD = stable disease; TTP = time to progression.

[a] Data based on local review.

[b] By palpation.

TAMRAD study

A GINECO (Groupe d'Investigateurs Nationaux pour l'Etude des Cancers de l'Ovaire et du Sein) randomized, phase II trial evaluated everolimus in combination with tamoxifen versus tamoxifen alone in patients with HR-positive, HER2-negative metastatic breast cancer with previous exposure to aromatase inhibitors (TAMRAD). Patients were randomly assigned according to primary or secondary hormone resistance. Primary resistance was defined as relapsing during or within 6 months of stopping adjuvant AI treatment or progressing within 6 months of starting AI treatment in the metastatic setting. Secondary resistance was defined as relapsing >6 months after stopping adjuvant AI treatment or responding for ≥6 months to AI treatment in the metastatic setting. Overall, the CBR was 42% with tamoxifen alone versus 61% with tamoxifen plus everolimus at median 13 months of follow-up. Median TTP was 4.5 months with tamoxifen alone versus 8.6 months with tamoxifen plus everolimus. Therefore, tamoxifen plus everolimus demonstrated significant improvement in CBR at 6 months compared with tamoxifen alone. In an exploratory analysis of primary and secondary hormone-resistant subgroups, everolimus combination therapy resulted in a median TTP of 5.4 months for patients with primary resistance compared with 14.8 months for those with acquired resistance. Similarly, CBR was greatest among patients receiving combination therapy with secondary resistance (74% compared with 48% for monotherapy), while patients with primary hormone resistance had only a slightly higher CBR with the addition of everolimus (46% compared with 36% for monotherapy).[40]

BOLERO-2 study

The promising results observed in phase II studies warranted further studies in patients with ER-positive, HER2-negative metastatic breast cancer and previous exposure to aromatase inhibitors. BOLERO-2 was a multinational, double-blind, placebo-controlled phase III study of postmenopausal women with ER-positive, HER2-negative locally advanced or metastatic breast cancer refractory to nonsteroidal aromatase inhibitors who had documented recurrence or progression. Individuals were stratified by sensitivity to previous hormonal therapy and the presence of visceral metastasis. Patients were then randomly assigned (2:1) to receive everolimus 10 mg daily or placebo orally once daily, with both arms receiving exemestane 25 mg daily. Treatment was continued until disease progression or unacceptable toxicity occurred. The primary outcome was PFS as assessed by the investigators. Secondary outcomes included overall survival, overall response rate, time to deterioration of Eastern Cooperative Oncology Group performance status, safety, and change in quality-of-life scores over time. Between June 2009 and January 2011, 724 patients were randomly assigned from 24 countries; 84% had hormone-sensitive disease and 56% had visceral disease.

A planned interim analysis of 359 PFS events determined that everolimus plus exemestane significantly improved PFS compared with exemestane alone (6.9 vs 2.8 months, respectively; hazard ratio = 0.43; 95% CI: 0.35–0.54, $P < 0.001$ [local assessment]).[37] Objective response rates were 9.5% with everolimus plus exemestane and 0.4% with exemestane alone ($P < 0.001$), and the percentages of patients with stable disease were 70.1% and 58.6%, respectively. A prespecified 12-month follow-up analysis determined that median PFS was 7.4 months versus 3.2 months (hazard ratio = 0.44; 95% CI: 0.36–0.53, $P < 0.001$ [local assessment]) and objective response rates were 12.0% and 1.3% for combination versus exemestane-only therapy, respectively ($P < 0.001$).[38] The clinical benefit rates for the combination versus exemestane-only therapy groups were 50.5% and 25.5%, respectively ($P < 0.001$). PFS rates were similar regardless of whether or not everolimus dose intensity during the study was reduced, possibly because of adverse events (<7.5 mg/day, hazard ratio = 0.40, 95% CI: 0.31–0.52; ≥7.5 mg/day, hazard ratio = 0.45, 95% CI: 0.37–0.56).[43] Recently, an 18-month follow-up analysis determined that the median PFS was 7.8 months versus 3.2 months (hazard ratio = 0.45; 95% CI: 0.38–0.54, $P < 0.001$ [local assessment]) and objective response

Table 2
PI3K/mTOR inhibitors being investigated for treatment of HR-positive advanced breast cancer.

Agents	Phase	Patient population	Treatment	Clinicaltrials.gov
mTOR inhibitors				
Ridaforolimus	II	ER+, HER2− advanced BC	Ridaforolimus + dalotuzumab vs exemestane vs ridaforolimus or dalotuzumab monotherapy	NCT01234857
	II	ER+, HER2− advanced BC	Ridaforolimus + dalotuzumab vs exemestane vs ridaforolimus + exemestane	NCT01605396
AZD2014	I	ER+ advanced BC	AZK2014 + fulvestrant	NCT01597388
PI3K inhibitors				
XL147	I/II	ER+, HER2− BC refractory to nonsteroidal AI	XL147 + letrozole	NCT01082068
BKM120	III	ER+, HER2− BC refractory to nonsteroidal AI	BKM120 + fulvestrant vs placebo + fulvestrant	NCT01610284
	I	HR+ advanced BC	BKM120 + letrozole Intermittent BKM120 + letrozole	NCT01248494
	I	ER+ stage IV BC	BKM120 + fulvestrant	NCT01339442
GDC-0941	II	HR+ advanced BC resistant to AI	GDC-0941 + fulvestrant vs placebo + fulvestrant	NCT01437566
Dual PI3K/mTOR inhibitors				
XL765	I/II	ER+, HER2− BC refractory to nonsteroidal AI	XL765 + letrozole	NCT01082068
BEZ235	I	HR+ advanced BC	BEZ235 + letrozole	NCT01248494
PF-04691502	II	ER+, HER2− early BC	PF-04691502 vs PF-04691502 + letrozole vs letrozole	NCT01430585
GDC-0980	II	HR+ advanced BC to AI	GDC-0980 + fulvestrant vs placebo + fulvestrant	NCT01437566

AI, aromatase inhibitor; BC, breast cancer; ER+, estrogen receptor–positive; HER2−, human epidural growth factor 2 receptor–negative; HR+, hormone receptor–positive.

rates were 12.6% and 1.7% for combination versus exemestane-only therapy, respectively ($P < 0.001$).[39] The clinical benefit rates for the combination versus exemestane-only therapy groups were 51.3% and 26.4%, respectively ($P < 0.001$).

Clinical considerations and future direction

While results from TAMRAD and BOLERO-2 demonstrated everolimus in combination with endocrine therapy to derive significant clinical benefit compared with endocrine monotherapy, no such benefit was observed with the combination of temsirolimus and letrozole in patients with breast cancer refractory to previous endocrine treatment. Although the exact causes of the differences in efficacy cannot be definitively determined, some potential causes may be due to stratification with respect to previous endocrine responsiveness and differences in dosing schedules. In the everolimus studies, patients unresponsive to previous endocrine therapy were included, whereas in the temsirolimus study, patients could have been either treated or not treated previously with endocrine therapy. Also, patients in the everolimus study were administered 10 mg daily throughout the trial, whereas those in the temsirolimus study were administered the drug for 5 days every 2 weeks, suggesting that continuous dosing may be necessary for effective mTOR inhibition.

Currently, new therapies that target the PI3K/mTOR pathway in HR-positive advanced breast cancer (Table 2) are being developed. The mTOR inhibitors ridaforolimus and AZD2014 are being evaluated in phase II clinical trials of ER-positive, HER2-negative advanced breast cancer. Drugs that selectively inhibit PI3K alone (XL147, BKM120, and GDC-0941) or both PI3K and mTOR (XL765, BEZ235, PF-04691502, GDC-0980) are also in development. Although the majority of these studies are in phase I and II development, BKM120 is currently in a phase III study in combination with fulvestrant in ER-positive, HER2-negative breast cancer refractory to nonsteroidal aromatase inhibitors.

Conclusion

mTOR signaling pathway activation plays a central role in the development of hormone therapy resistance in breast cancer. Use of mTOR inhibitors, including everolimus, has been shown to increase efficacy of aromatase inhibitors and significantly improve PFS in patients with HR-positive metastatic breast cancer. Ongoing and future studies are focusing on assessing the benefit of combining everolimus with other endocrine agents and the development of other inhibitors of the PI3K/mTOR pathway for treating HR-positive advanced breast cancer. Considering the multiple pathways involved in the HR network, targeting other components of pathologically activated intracellular signaling in breast cancer may prove to be a new direction in clinical research.

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Conflict of interest statement

Dr Shtivelband participated as an investigator for the BOLERO-2 study. He has no conflicts of interest to disclose.

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