Role of mTOR Inhibition in Preventing Resistance and Restoring Sensitivity to Hormone-Targeted and HER2-Targeted Therapies in Breast Cancer

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Abstract: Even with hormone-targeted and human epidermal growth factor receptor 2 (HER2)–targeted anticancer agents, intrinsic resistance or acquired resistance are common occurrences in estrogen receptor–positive and HER2-positive breast cancers, respectively. Potential mechanisms for resistance to targeted agents include steric inhibition imposed by other cellular elements, molecular changes in the target receptor, alterations in the regulation of downstream signaling components, compensatory cross-talk with other signaling pathways, and pharmacogenetic alterations in the host. Evidence suggests that both hormone receptor–positive tumors and HER2-overexpressing tumors use the phosphoinositide 3-kinase/Akt/ mammalian target of rapamycin (mTOR) pathway to escape control of anti-hormone and anti-HER2 therapies. The combination of mTOR inhibitors with hormone-targeted or HER2-targeted therapies appears to be a promising strategy for overcoming resistant disease and preventing the development of resistance.

Introduction

The development of new strategies for the treatment of breast cancer has focused not only on target identification but also on understanding the expression, regulation, and function of critical signaling pathways involved in cancer initiation and progression. This process allowed the identification of breast cancer subsets with distinct biology, as well as the development of targeted therapies. Notable examples are the successful use of endocrine therapy (selective estrogen-receptor [ER] modulators, such as tamoxifen and fulvestrant [Faslodex, AstraZeneca], or aromatase inhibitors, such as anastrozole [Arimidex, AstraZeneca], letrozole [Femara, Novartis], and exemestane [Aromasin, Pfizer]) for women with hormone-sensitive (ER-expressed or progesterone receptor [PR]–expressed) tumors, and the use of anti–human epidermal growth
factor receptor 2 (HER2) therapy (trastuzumab [Herceptin, Genentech] and lapatinib [Tykerb, GlaxoSmithKline]) for women with HER2-overexpressing tumors. Breast cancer therapy is therefore currently guided by clinical staging as well as analysis of biological features of the tumor, such as hormonal receptor expression or HER2 overexpression. Despite the use of agents that target specific aberrations in breast cancer cells, many patients with hormone receptor–positive or HER2-positive tumors are intrinsically resistant to hormone-targeted or HER2-targeted therapies, and many patients will eventually acquire resistance to these treatments after an initial response. Intrinsic resistance is evidenced by the lower objective response rate (ORR) achieved with these agents upfront. Acquired resistance may occur as tumor cells adapt to the stress of treatment by using alternate cellular signaling pathways. Potential mechanisms for resistance to targeted agents include...
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The objectives of this article are to review the involvement of the mammalian target of rapamycin (mTOR) pathway in these resistance mechanisms and to summarize preclinical and clinical data suggesting the promise of combination therapy with mTOR inhibitors in preventing resistance and restoring sensitivity to hormone-targeted and HER2-targeted agents.

PI3K/Akt/mTOR Signaling Pathways in Breast Cancer

The mTOR is an intracellular serine/threonine kinase that is positioned downstream of phosphoinositide 3-kinase (PI3K)/Akt (Figure 1) and is dysregulated in a variety of cancers.8,9 The target of rapamycin (TOR) plays a central role in cell growth regulation by integrating signals from growth factors, nutrients, and cellular energy levels. TOR forms 2 distinct physical and functional complexes, termed TOR complex 1 (TORC1) and TOR complex 2 (TORC2). TORC1, which is sensitive to rapamycin, regulates translation and cell growth, whereas TORC2, which is insensitive to rapamycin, regulates cell morphology and cell growth.10 Both genetic and biochemical data suggest that activation of the PI3K/Akt/mTOR pathway contributes to breast cancer development and tumorigenesis.11 Breast cancer predisposition is found in patients with familial syndromes (eg, Cowden disease, Bannayan-Zonana syndrome) characterized by germline mutations in phosphatase and tensin homolog (PTEN), a negative regulator of the PI3K pathway.12-13 Molecular alterations involving the PI3K/Akt pathway occur in more than 30% of invasive breast tumors. Alterations in breast cancer resulting in hyperactivity of the PI3K pathway include gain-of-function mutations in PIK3CA (the gene encoding the PI3K catalytic subunit p110α), mutations in AKT1, amplifications of AKT2, and loss of PTEN.14 Mutations in PIK3CA cluster in 2 major “hot spots,” located in the helical (E542K and E545K in exon 9) and catalytic (H1047R in exon 20) domains.14-16 Expression of these mutant p110α isoforms confers growth factor–independent proliferation and protection from anoikis (a form of cell death) and chemotherapy. PI3K catalytic subunit alpha (PIK3CA) mutations in primary breast tumors have been associated with lymph node metastases and the presence of ER, PR, and HER2 overexpression.14,15,17

The impact of these mutations and of PTEN loss on the pathogenesis of breast cancer and patient outcome is not yet completely clear. Indeed, 2 recent articles suggest that breast cancers with PIK3CA mutations exhibit lower PI3K pathway activation and a better prognosis.17,18 On the other hand, PI3K hyperactivity has been associated with resistance to anti-HER receptor therapies, and it is generally accepted that anti-HER2 therapies inhibit PI3K/Akt signaling downstream of the HER2 receptor pathway in order to inhibit tumor growth.19-21 Consistent with this activity, the presence of activating PIK3CA mutations and loss of PTEN in HER2-overexpressing cancers correlates with a lower response to trastuzumab.19-21 These data also suggest that inhibitors of the PI3K pathway, currently in clinical development, might be used to reverse acquired and de novo drug resistance. Overactivity of the Akt pathway is involved in endocrine resistance, modulating responses to signals communicated through the ER. It is also known that breast cancer cell lines with activated Akt (eg, via loss of the PTEN suppressor gene) are especially sensitive to mTOR antagonists.22

Preventing Resistance and Restoring Sensitivity With Combination Therapy: mTOR Inhibitors

Because evidence suggests that both hormone receptor–positive tumors and HER2-overexpressing tumors use the PI3K/Akt/mTOR pathway to escape control of antihormone and anti-HER2 therapies, combination therapy with mTOR inhibitors is a rational approach to determine whether resistance to these agents can be prevented and sensitivity can be restored with the addition of mTOR inhibition. Rapamycin (sirolimus [Rapamune, Pfizer] was the first agent found to partially inhibit mTOR23 and is currently approved for the prevention of kidney transplant rejection. Everolimus (Afinitor, Novartis) and temsirolimus (Torisel, Pfizer) are mTOR inhibitors approved by the US Food and Drug Administration (FDA) for the treatment of advanced renal cell carcinoma.24,25 Everolimus has also been approved for adults with unresectable, locally advanced, or metastatic progressive neuroendocrine tumors of pancreatic origin, adults with renal angiomyolipoma and tuberous sclerosis complex not requiring immediate surgery, and adults and children ages 3 years and older with subependymal giant cell astrocytoma associated with tuberous sclerosis complex who require therapeutic intervention but are not candidates for curative surgical resection.25 Recently, everolimus was approved for the treatment of postmenopausal women with advanced hormone receptor–positive, HER2-negative breast cancer in combination with exemestane after failure of treatment with letrozole or anastrozole.25 Other mTOR inhibitors and dual PI3K/mTOR inhibitors are in development (eg, ridaforolimus, NVP-BEZ235, XL765) for a variety of solid tumors, including breast cancer.
Hormone Therapies Plus mTOR Inhibitors

Preclinical and patient data suggest that aberrant activation of the PI3K/Akt/mTOR pathway may have a role in resistance to hormone-targeted therapies. This pathway has been shown by proteomic and gene expression profiling to be upregulated in MCF-7 human breast cancer cells subjected to long-term estrogen deprivation.26 Reverse-phase protein microarray analysis of patient breast tumor samples revealed a protein signature of PI3K pathway activation associated with poor outcomes after adjuvant endocrine therapy.26 Overactive growth factor receptor signaling is thought to mediate resistance to hormone-targeted therapies; cross-talk between the ER pathway and the PI3K/Akt/mTOR pathway and between the ER pathway and the HER2 pathway is a proposed mechanism of endocrine therapy resistance.25 In long-term estrogen-deprived MCF-7 human breast cancer cells, activation of the insulin-like growth factor-1 (IGF-1) receptor and the insulin receptor was associated with PI3K pathway hyper-activation.26 Activation of the PI3K pathway by EGF or IGF-1 promoted ERα transcription in MCF-7 cells.27

Support for the combination of mTOR inhibitors with either tamoxifen or an aromatase inhibitor has been demonstrated in preclinical models of ER-positive hormone-sensitive and hormone-resistant breast cancer.28 MCF-7 cells expressing a constitutively active Akt were able to proliferate under reduced estrogen conditions and were resistant to the growth inhibitory effects of tamoxifen, both in vitro and in vivo.28 However, cotreatment with temsirolimus inhibited mTOR activity and restored sensitivity to tamoxifen—primarily through induction of apoptosis—thus suggesting that Akt-induced tamoxifen resistance may be mediated in part by signaling through the mTOR pathway. In vitro29 and in vivo30 models have shown that everolimus restores sensitivity of cancer cells to letrozole. Beeram and colleagues30 reported on the use of an mTOR kinase inhibitor to reverse endocrine resistance in a breast cancer model in vivo. The authors generated an MCF-7 cell line with constitutively activated Akt that demonstrated resistance to the aromatase inhibitors letrozole and fulvestrant, and they showed that cotreatment with everolimus restored sensitivity of the breast cancer cell lines to both endocrine agents.

Preclinical studies support the efficacy of combining hormonal- and mTOR-targeted therapies in resistant breast cancer. The combination of rapamycin and tamoxifen restored sensitivity to tamoxifen in tamoxifen-resistant breast cancer cells with high Akt activity, and the combination of temsirolimus and tamoxifen restored sensitivity to tamoxifen in tamoxifen-resistant mouse xenografts with high Akt activity.28 In breast cancer cells resistant to both tamoxifen and fulvestrant, reversal of resistance by rapamycin was associated with increased expression of ERα protein and modification of the phosphor-ser-167 ERα/total ERα ratio, and combination therapy with rapamycin and fulvestrant restored 40% of the fulvestrant gene-expression signature.31 Everolimus showed additive/synergistic effects with letrozole in breast cancer cell lines modeling endocrine-sensitive29,32 and endocrine-resistant disease.32 In breast cancer cellular models of letrozole, fulvestrant, and tamoxifen resistance, low concentrations of everolimus restored responses to these agents.30 Additive growth inhibitory effects of everolimus and tamoxifen were observed in MCF-7 breast cancer cells,33 and everolimus showed anti-proliferative activity in tamoxifen-resistant breast cancer cells.32

As a result of promising preclinical data supporting the efficacy of combining hormone- and mTOR-targeted therapies in resistant breast cancer, this combination has been evaluated in clinical trials. A phase II, 3-arm study evaluated daily letrozole alone or in combination with daily temsirolimus (10 mg/day or 30 mg/day for 5 days every 2 weeks) in postmenopausal women with locally advanced or metastatic breast cancer.34 The median progression-free survival (PFS) was 11.5 months in the letrozole 2.5 mg/temsirolimus 10 mg arm, 13.2 months in the letrozole 2.5 mg/temsirolimus 30 mg arm, and 11.6 months in the letrozole 2.5 mg alone arm.34 The combination was overall well tolerated. However, results from a phase III randomized, double-blind, multicenter clinical trial of temsirolimus plus letrozole 2.5 mg/day versus placebo plus letrozole in postmenopausal women with newly diagnosed locally advanced or metastatic breast cancer showed that both treatment arms had virtually identical median PFS and ORRs, resulting in early discontinuation of the study.35

In a phase I trial of 18 patients with advanced breast cancer, everolimus in combination with letrozole demonstrated antitumor activity, with a durable complete response exceeding 22 months in 1 patient, stable disease in 9 patients, and stable disease exceeding 12 months in 4 patients.36 The combination was well tolerated.36 There was also a trend of increased efficacy (higher decrease in Ki67) for the everolimus/letrozole combination versus letrozole alone in a neoadjuvant phase II trial for patients with ER-positive breast cancer.37

Results from the phase II TAMRAD (Tamoxifen and RAD001) study conducted in postmenopausal patients with aromatase inhibitor–resistant, ER-positive, metastatic breast cancer found that the addition of everolimus to tamoxifen almost doubled time to progression (4.5 months vs 8.6 months for tamoxifen alone and tamoxifen-everolimus, respectively; hazard ratio [HR], 0.54; 95% confidence interval [CI], 0.36–0.81; P = .002), corresponding to a 46% reduction in risk of progression with combination therapy.38 Interestingly, a subgroup analysis
revealed that for the tamoxifen-everolimus arm, this risk reduction was greatest among patients with secondary resistance to endocrine treatment (54%). Higher clinical benefit rates (defined as the absence of progression at 6 months) were also observed in patients with secondary hormone resistance who received combination treatment versus tamoxifen alone (74% vs 48%, respectively). The safety profile was consistent with adverse events previously reported for everolimus, with stomatitis being the most commonly reported grade 3/4 adverse events in the combination arm.

The benefit of everolimus in aromatase inhibitor–resistant advanced breast cancer has been further supported by data from the phase III BOLERO-2 (Breast Cancer Trials of Oral Everolimus) study comparing the combination of exemestane and everolimus with exemestane alone in patients with ER-positive breast cancer. Among the 724 patients enrolled in this trial, 84% had demonstrated previous sensitivity to endocrine treatment; more than 50% had received 3 or more previous therapies. At interim analysis according to local assessment, the addition of everolimus to exemestane significantly prolonged median PFS by 4.1 months versus exemestane alone (6.9 months vs 2.8 months, respectively; \( P < 0.001 \)), and the clinical benefit rate was 33% among patients receiving combination treatment compared with 18% for exemestane only. A longer-term prespecified 12-month follow-up analysis of BOLERO-2 was recently presented. Median PFS was 7.4 months versus 3.2 months for combination treatment and exemestane only, respectively (\( P < 0.001 \)). Clinical benefit rates were 50.5% (combination treatment) and 25.5% (exemestane only). The median dose intensity (cumulative dose/duration of exposure) of everolimus was 8.6 mg (range, 0.3–10 mg), and PFS improvements were maintained in patients whose dose intensity during the study was lower, potentially due to adverse events (<7.5 mg/day: HR, 0.40; 95% CI, 0.31–0.52 vs ≥7.5 mg/day: HR, 0.45; 95% CI, 0.37–0.56). At 18 months of follow-up, the benefit of everolimus plus exemestane was maintained and resulted in a 4.6-month prolongation in median PFS. Median PFS was 3.2 months for placebo and 7.8 months for everolimus plus exemestane (\( P < 0.0001 \)). Taken altogether, these data led to the recent FDA approval of everolimus for the treatment of postmenopausal women with advanced breast cancer, potentially foreshadowing a shift in the treatment paradigm of breast cancer.

**Anti-HER2 Therapies Plus mTOR Inhibitors**

Aberrant activation of the PI3K pathway is also thought to be involved in anti-HER2 therapy resistance. Resistance may be related to loss or dysregulation of PTEN. A large-scale RNA interference genetic screen of 8,000 genes identified only PTEN suppression as a mediator of trastuzumab resistance in HER2-overexpressing breast cancer cells. In tumor xenografts in athymic nude mice, inhibition of PTEN expression by injection of PTEN anti-sense oligonucleotides conferred resistance to trastuzumab. An analysis of PTEN expression levels and association with treatment response in tumor samples from breast cancer patients who subsequently received trastuzumab–based therapy revealed that patients with PTEN-deficient tumors had significantly lower ORR than those with PTEN-positive tumors. PI3K pathway activation due to mutations in PIK3CA or low PTEN was associated with shorter PFS in tumor samples from trastuzumab-treated breast cancer patients. Trastuzumab-resistant BT474 cells generated by continuous culture of previously sensitive cells in a trastuzumab-containing medium have elevated levels of phosphorylated Akt and Akt kinase activity compared with the BT474 parental cell line. These resistant cells also showed increased sensitivity to PI3K inhibitors.

Based on promising clinical results in patients with refractory breast cancer, the mTOR inhibitors ridaforolimus, sirolimus, and temsirolimus are currently being investigated in combination with other agents in phase II and III clinical trials (Table 1). Everolimus is currently in phase III clinical trials in combination with vinorelbine and trastuzumab in locally advanced or metastatic HER2-positive breast cancer resistant to trastuzumab and previously treated with a taxane (BOLERO-3, NCT01007942). Despite promising clinical results,
inhibition of mTOR results in induction of insulin receptor substrate–1 expression, causing a paradoxical Akt activation both in cancer cell lines and in patient tumors treated with mTOR inhibitors.50 IGF-1 receptor inhibition prevents rapamycin-induced Akt activation and sensitizes tumor cells to inhibition of mTOR. In contrast, IGF-1 reverses the anti-proliferative effects of rapamycin.50 Similar inhibition of activated Akt induction was achieved with a PI3K inhibitor, LY294002, implying that the phenomenon is PI3K dependent.50 These data suggest that feedback downregulation of receptor tyrosine kinase signaling is a frequent event in tumor cells with constitutive mTOR activation.50 Reversal of this feedback loop by rapamycin and its analogs (temsirolimus, everolimus, and deforolimus), which target only TORC1—could actually negate this recognized feedback mechanism of resistance, since TORC2 can activate Akt, which is also downstream of PI3K. Therefore, a strategy that ablates mTOR function and prevents Akt activation may have improved antitumor activity. Consistent with this rationale, the efficacy of XL765, a dual inhibitor of PI3K and mTOR, is being assessed in combination with letrozole in a phase I/II trial of patients with refractory breast cancer (NCT01082068).51 Additionally, a phase Ib study of BKM120 (PI3K inhibitor) or BEZ235 (PI3K/mTOR inhibitor) in combination with letrozole is being conducted in postmenopausal women with ER-positive metastatic breast cancer (NCT01248494).52 The safety and efficacy of a novel PI3K inhibitor (XL147) administered in combination with trastuzumab or with paclitaxel and trastuzumab are being evaluated in patients with HER2-

### Table 1. Ongoing Clinical Trials of mTOR Inhibitors in Resistant Breast Cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Phase</th>
<th>Setting (NCT ID)</th>
<th>Treatment Arms</th>
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<tr>
<td>Everolimus</td>
<td>I/II</td>
<td>HER2-positive mBC, trastuzumab-resistant (NCT00458237)</td>
<td>Everolimus + trastuzumab</td>
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<td>I/II</td>
<td>HER2-overexpressing BC, trastuzumab-resistant (NCT00317720)</td>
<td>Everolimus + trastuzumab</td>
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<td>I/II</td>
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<td></td>
<td>II</td>
<td>Advanced/mBC after aromatase inhibitor failure (NCT00570921)</td>
<td>Everolimus + fulvestrant</td>
<td>44</td>
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<tr>
<td></td>
<td>II</td>
<td>ER-positive mBC, hormone refractory (NCT00912340)</td>
<td>Everolimus + trastuzumab Trastuzumab</td>
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<td>III</td>
<td>HER2-positive locally advanced BC/mBC, trastuzumab-resistant (BOLERO-3, NCT01007942)</td>
<td>Everolimus + vinorelbine + trastuzumab Vinorelbine + trastuzumab</td>
<td>572</td>
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<td>III</td>
<td>ER-positive, locally advanced BC/mBC refractory to letrozole or anastrozole (BOLERO-2, NCT00863655)</td>
<td>Everolimus + exemestane Placebo + exemestane</td>
<td>724</td>
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<td>Ridaforolimus</td>
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<td>ER-positive BC, endocrine-resistant (NCT01234857)</td>
<td>Ridaforolimus + dalotuzumab Exemestane Ridaforolimus Dalotuzumab</td>
<td>352</td>
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<tr>
<td>Sirolimus</td>
<td>II</td>
<td>HER2-positive mBC, trastuzumab-resistant (NCT00411788)</td>
<td>Sirolimus + trastuzumab</td>
<td>30</td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>I/II</td>
<td>HER2-amplified or triple-negative mBC, trastuzumab-resistant (NCT01111825)</td>
<td>Temsirolimus + neratinib</td>
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<td></td>
<td>I/II</td>
<td>mBC/locally recurrent BC (NCT006999491)</td>
<td>Temsirolimus + cixutumumab</td>
<td>68</td>
</tr>
<tr>
<td>XL765</td>
<td>I/II</td>
<td>HR-positive and HER2-negative BC refractory to a nonsteroidal aromatase inhibitor (NCT01082068)</td>
<td>XL765 + letrozole XL765 + letrozole XL147 + letrozole</td>
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</table>

*Anticipated enrollment.

BC=breast cancer; BOLERO=Breast Cancer Trials of Oral Everolimus; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; mBC=metastatic breast cancer.
positive metastatic breast cancer that progressed during previous trastuzumab treatment (NCT01042925).  

Conclusion

Resistance to hormone-targeted and HER2-targeted therapies is a common problem in the treatment of patients with breast cancer. Knowledge of signaling pathways that potentially mediate resistance is useful when designing approaches to prevent or reverse resistance. Aberrant mTOR activity is thought to be involved in resistance to both hormone-targeted and HER2-targeted therapies. The combination of mTOR inhibitors with hormone-targeted or HER2-targeted therapies appears to be a promising strategy for overcoming resistant disease and preventing the development of resistance. Phase II and III clinical trials evaluating the efficacy and safety of combining these targeted agents are in progress. The overall goal of the combinations under investigation is to increase the magnitude and duration of the response to treatment while maintaining safety and tolerability.

In the future, it will be important to design combination treatment trials with patient populations stratified based on previous treatment exposure and/or sensitivity and to identify tumor phenotypes in patients whose tumors respond to a given treatment versus the ones that do not respond. Somatic DNA alterations such as PIK3CA and AKT1 mutations, PTEN loss, or PI3K activating oncogene amplification identify cancers with aberrant PI3K activation and potential dependence on the PI3K pathway. This correlation may be an important consideration in the selection of patients for trials with PI3K/mTOR inhibitors, although whether the activity of PI3K pathway inhibitors will be more effective in these tumors remains to be determined.

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