mTOR inhibitors in the management of hormone receptor-positive breast cancer: the latest evidence and future directions

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Received 18 December 2011; revised 6 March 2012; accepted 7 March 2012

Background: There is an unmet therapeutic need in endocrine-resistant, hormone receptor (HR)-positive, human epidermal growth factor receptor 2-negative advanced breast cancer (BC). Preclinical studies support the hypothesis that the mammalian target of rapamycin (mTOR) inhibition could potentially overcome resistance to endocrine therapy.

Materials and methods: A literature review regarding BC and mTOR inhibitors was undertaken. The reference lists from retrieved manuscripts were reviewed to identify further studies.

Results: Phase II studies have reported that the combination of mTOR inhibitors with endocrine therapy shows efficacy in patients with advanced disease that progressed after treatment with aromatase inhibitors. The recent findings of the phase III BOLERO-2 confirmed that everolimus in combination with exemestane significantly improved progression-free survival and response rate, with a manageable safety profile.

Conclusions: The addition of everolimus to exemestane for women with HR-positive metastatic BC is now considered a new therapeutic strategy. However, a word of caution should be added regarding toxic effects, which might limit practical use and compliance. It is essential that clinicians are educated about key recommendations for toxicity management and specific guideline dose modifications. Additional research efforts with the addition of these compounds in the early-stage setting is greatly needed to improve the survival of patients with HR-positive BC.

Key words: breast cancer, endocrine resistance, everolimus, mTOR inhibitors, temsirolimus

introduction

Approximately three quarters of all invasive breast tumors are estrogen receptor (ER)- and/or progesterone receptor (PR)-positive, including at least half of all cancers in premenopausal women [1]. The natural history of hormone receptor (HR)-positive disease differs from that of HR-negative disease in terms of time to recurrence, site of recurrence, and overall aggressiveness of the disease. Compared with patients with ER-negative tumors, patients with ER-positive tumors experience a relatively constant hazard of recurrence over time [2, 3]. In women treated with tamoxifen for 5 years, more than half of all recurrences occur in years 6–15 after diagnosis [4]. Although tamoxifen and aromatase inhibitors (AI) lower the risk of recurrence for several years after they are stopped, late recurrences and deaths remain a major clinical challenge. In the metastatic setting, there are some patients with HR-positive disease who have durable response to antiestrogen therapy, although the majority of patients will have a short survival of <3 years. This review will focus on the management of HR-positive breast cancer (BC), the current standard of care, and the new evidence on use of mammalian target of rapamycin (mTOR) inhibitors in this setting.
current management of HR-positive early BC

The efficacy of adjuvant tamoxifen for women with ER-positive early BC has been clearly demonstrated (supplemental Appendix S1, available at Annals of Oncology online). Adjuvant tamoxifen treatment has been associated with a 31% reduction in the annual BC mortality rate among HR-positive women with early BC [4], making it a standard of care for this patient population. Guidelines suggest that selected patients could be treated with tamoxifen alone, especially those with low risk of recurrence [5–7]. However, with the advent of nonsteroidal AI—anastrozole and letrozole—and steroidal AI—exemestane—the standard of care has been evolving. AIs have demonstrated improved activity compared with tamoxifen for the adjuvant endocrine treatment of postmenopausal patients with HR-positive BC. AIs have been evaluated in different adjuvant endocrine settings: as upfront therapy, switch to an AI after 2–3 years of tamoxifen or extended therapy following 5 years of tamoxifen.

The various studies are consistent in demonstrating that the use of a third-generation AI in postmenopausal women with HR-positive BC lowers the risk of recurrence, including ipsilateral breast tumor recurrence, contralateral BC, and distant metastatic disease, compared with tamoxifen alone when the AI is used as initial adjuvant therapy, sequential therapy, or extended therapy. Thus, current international guidelines recommend that postmenopausal women with early BC receive an AI as initial adjuvant therapy, sequential with tamoxifen, or as extended therapy in those situations where endocrine therapy is to be utilized [7–9].

first-line endocrine therapy for MBC

aromatase inhibitors. Tamoxifen was established in the treatment of hormone-responsive metastatic breast cancer (MBC) based upon superior response and duration and favorable toxicity, when compared in randomized trials to high-dose estrogens, androgens, progestins, and the AI, aminoglutethimide, in postmenopausal patients (supplemental Appendix S2, available at Annals of Oncology online). The likelihood of response to tamoxifen is 65% in ER- and PR-positive tumors, 30% in ER- or PR-positive ones, and < 5% in both ER- and PR-negative tumors [10]. Tamoxifen has been recently displaced by third-generation AIs as first-line treatment of advanced HR-positive MBC, although double-blinded crossover trials showed no difference for either sequence in patients exposed to both treatments [11].

Studies comparing tamoxifen versus AI in the first-line metastatic setting were largely conducted at a time when adjuvant AI use was uncommon. Two phase III double-blind trials compared tamoxifen versus anastrozole in the first-line setting for postmenopausal MBC [12, 13]. AI was superior to tamoxifen only in those patients with positive HR, with an advantage in median progression-free survival (PFS) (10.7 versus 6.4 months, P = 0.022). A third trial showed a significant improvement in median time to progression (TTP) and overall survival (OS) in the anastrozole compared with the tamoxifen group [18.0 versus 7.0 months, hazard ratio = 0.13, P < 0.01 and 17.4 versus 16.0 months, hazard ratio = 0.64, P = 0.003, respectively] [14].

A single phase III study that compared letrozole versus tamoxifen in the first line setting showed a benefit in PFS compared with tamoxifen (9.4 versus 6.0 months) [15]. Prospectively planned analyses of the intent-to-treat population showed that letrozole significantly improved OS compared with tamoxifen over the first 24 months of the trial.

Exemestane has also been studied in the first-line treatment in the metastatic setting, and a phase III trial showed superior PFS to tamoxifen (9.9 versus 5.8 months); however, this did not translate to a longer term benefit in OS [16].

Two meta-analyses of randomized trials of AIs compared with other endocrine therapy as first-line therapy showed a significantly superior OS [hazard ratio = 0.89, 95% confidence interval (CI) 0.8–0.9] favoring treatment with a third-generation AI [17, 18].

fulvestrant. Fulvestrant is an ER antagonist that has no agonist effects. As first-line therapy, fulvestrant (250 mg as a monthly injection, without the initial loading dose) has been compared with tamoxifen in a phase III non-inferiority trial [19]. The non-inferiority of fulvestrant was not established (hazard ratio = 1.18, 95% CI 0.98–1.44). A loading dose regimen was developed in order to produce a steady-state concentration of fulvestrant. The CONFIRM trial showed the superiority of high-dose fulvestrant (fulvestrant 500 mg monthly after the loading schedule versus fulvestrant 250 mg monthly) [20]. These results prompted the Food and Drug Administration approval of fulvestrant 500 mg.

FIRST is a phase II trial that evaluated fulvestrant 500 mg versus anastrozole as first-line treatment of HR-positive advanced BC [21]. Fulvestrant improved TTP compared with anastrozole (23.4 versus 13.1 months), (hazard ratio = 0.66; 95% CI 0.5–0.9).

second-line endocrine therapy for MBC

aromatase inhibitors. A lack of complete cross-resistance between steroidal and nonsteroidal AIs is supported by several studies showing clinical benefit (objective response or stable disease for >24 weeks) with exemestane after previous nonsteroidal AIs [22]. The opposite sequence was also investigated in patients receiving exemestane as first-line endocrine treatment: when crossed over to letrozole (n = 17) or anastrozole (n = 1) at the time of progression, 55.6% obtained a clinical benefit [23].

fulvestrant. As second-line and subsequent therapy, fulvestrant (250 mg monthly, without the initial loading dose) appears to be as effective as anastrozole in postmenopausal patients with advanced tamoxifen-resistant BC, with no difference in TTP or OS [24–26]. Fulvestrant has also been compared with exemestane in patients whose BC recurred after prior AI therapy in the EFFECT trial [27]. Here too, there was no significant difference between fulvestrant and exemestane for median TTP or OS.
mechanisms of resistance to antiestrogen treatment

The classic mechanism of action of ER is its nuclear function, also referred to as genomic activity, to alter the expression of genes important for normal cellular function and tumor growth and survival. The ER signaling pathway is also regulated by membrane receptor tyrosine kinases, including epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and insulin-like growth factor receptor (IGF-1R) [28]. This activation of ER by growth factor receptor signaling is referred to as ligand-independent receptor activation. These membrane kinases activate signaling pathways that eventually result in phosphorylation of ER as well as its coactivators and corepressors at multiple sites to influence their specific functions [29].

De novo and acquired resistance to endocrine therapy is a major clinical problem in the treatment of BC. Evidence is emerging to suggest both genomic and nongenomic mechanisms for cross talk in endocrine resistance despite the presence of tamoxifen or AI. Different mechanisms are involved when BC cells adapt themselves to escape from the manipulations blocking the ER signaling, which includes EGFR/HER2, mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) 1/2, and phosphatidylinositol-3-kinase/protein kinase B (Akt) pathways [30]. Estrogen-independent growth properties are mediated at least in part through the PI3K/Akt/mTOR pathway and that hyperactivation of this pathway account for survival of cells despite the presence of continued endocrine blockade.

Figure 1. PI3K/Akt/mTOR pathway and endocrine genomic and non-genomic cross talk. The PI3K/Akt/mTOR signaling network regulates proliferation, migration, cell survival, metabolism, and apoptosis. This network is dysregulated in BC enhancing translation and cellular proliferation. The mTOR proteins regulate activities of the translational regulators 4E-BP1 and S6K. mTOR-activated kinase S6K1 phosphorylates and destabilizes the insulin-receptor substrate 1 and 2 (IRS1 and IRS2). mTOR2 functions as an upstream regulator of Akt and delivers an additional stimulatory signal to mTOR1. Bi-directional cross talk between ER and growth factor receptors (e.g. HER2) mediate signaling via PI3K/Akt and MAPK pathways. These two pathways can directly phosphorylate genomic ER resulting in enhanced estrogen-regulated gene transcription. BC, breast cancer; ER, estrogen receptor; mTOR mammalian target of rapamycin; PI3k, phosphatidylinositol; PTEN, phosphatase and tensin homolog; S6K1, ribosomal protein S6 kinase; 4EBP1, 4E-binding protein.
The mTOR pathway

The mTOR is a serine/threonine protein kinase and it is placed downstream of the PI3K/Akt pathway (Figure 1). The mTOR pathway is mainly involved in the regulation of cell growth and proliferation by controlling these processes at the translational level. It has two main downstream messengers: the ribosomal p70 S6 kinase (S6K1) and the eukaryotic translation initiation factor 4E-binding protein (4E-BP1) [31]. Both proteins are translational activators critical for ribosome biogenesis and translation, including the synthesis of proteins necessary for cell cycle progression. In addition to its effect on protein translation mediated by S6K1 and 4E-BP1, mTOR activation leads to the phosphorylation of several downstream effectors and transcription factors.

The PI3K/Akt signaling pathway is dysregulated in a large number of human cancers, which in turn upregulates the downstream mTOR pathway [32]. Mutations in the catalytic domain of PI3K have been identified in 20%–25% of BCs [32, 33]. Furthermore, 15%–35% of patients with BC have a reduced expression of PTEN (phosphatase and tensin homolog deleted on chromosome 10), an endogenous inhibitor of the PI3K/AKT pathway [34].

Direct blockade of the mTOR pathway is a new and intriguing area in BC therapy, with the potential to modulate growth factor- and estrogen-dependent and estrogen-independent pathways, which contribute to the pathogenesis and progression of breast tumors.

**mTOR inhibitors in HR-positive BC**

**preclinical data**

Preclinical studies have shown that BC cells with upregulated Akt signaling are resistant to hormonal therapy, but sensitivity may be restored by treatment with mTOR inhibitors [35, 36]. Moreover, in models of estrogen-responsive BC, subnanomolar everolimus concentrations reduced the growth of BC cells in vitro, and enhanced antitumor activities were observed in combination with the AI, letrozole [37].

**mTOR inhibitors—neoadjuvant setting**

The safety and efficacy of everolimus as monotherapy was first evaluated in a preoperative pilot study in 31 postmenopausal patients with early BC (Table 1) [38]. Treatment with everolimus resulted in a significant 74% mean reduction in Ki67 from baseline \((P = 0.019)\). The p-S6 staining was significantly reduced independently of Ki67 \((P < 0.001)\). No data were reported on pathological response rate in these patients, which was not an end point in this pilot study.

Baselga et al. [39] conducted a randomized, double-blinded phase II trial in 270 postmenopausal women with operable ER-positive BC. Patients were randomly assigned to receive 4 months of neoadjuvant treatment with letrozole (2.5 mg/day) and either everolimus (10 mg/day) or placebo. The primary end point was clinical response by palpation. Biopsies were obtained at baseline and after 2 weeks of treatment. Response rate (RR) in the everolimus arm was higher than that with placebo (68% versus 59%; \(P = 0.062\); one-sided \(\alpha = 0.1\) level). Reductions in phospho-S6 were seen only in the everolimus

**Table 1. Clinical studies of mTOR inhibitors in HR-positive BC in the neoadjuvant setting**

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Study design</th>
<th>Patients</th>
<th>Treatment</th>
<th>Primary endpoint</th>
<th>Percent reduction in Ki67 in biopsies at diagnosis and surgery</th>
<th>Response rate by clinical palpation</th>
<th>Response (everolimus versus placebo): CR: 13% versus 9.1%; PR: 55 versus 50%; No change: 125 versus 30%; PD: 4.3 versus 9.8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaskill et al., 2011 [38]</td>
<td>31</td>
<td>One arm</td>
<td>Prognostic, operable</td>
<td>Everolimus (5 mg daily) for 14 days before surgery</td>
<td>Percent reduction in Ki67</td>
<td>Everolimus resulted in significant reduction in Ki67 (74%) from baseline ((P = 0.019))</td>
<td>Response (everolimus vs baseline)</td>
<td>CR: 13% versus 9.1%; PR: 55 versus 50%; No change: 125 versus 30%; PD: 4.3 versus 9.8%</td>
</tr>
<tr>
<td>Baselga et al., 2009 [39]</td>
<td>270</td>
<td>Double blind, placebo-controlled, multicenter USA/Europe</td>
<td>Postmenopausal, with previously untreated ER + BC, eligible for neoadjuvant therapy</td>
<td>Neoadjuvant Letrozole + Everolimus versus Neoadjuvant Letrozole + Placebo for 4 months</td>
<td>Response rate by clinical palpation</td>
<td>Response rate by clinical palpation</td>
<td>Response rate by clinical palpation</td>
<td>Response rate by clinical palpation</td>
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</tbody>
</table>

BC, breast cancer; ER, estrogen receptor; mTOR, mammalian target of rapamycin; CR, complete response; PR, progressive disease.
### Table 2. Clinical studies of mTOR inhibitors in HR-positive BC in the metastatic setting

<table>
<thead>
<tr>
<th>Reference</th>
<th>Trial/Phase</th>
<th>n</th>
<th>Study design</th>
<th>Patients</th>
<th>Treatment</th>
<th>Primary end point</th>
<th>Response/Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baselga, et al., 2005 [42]</td>
<td>Phase II</td>
<td>92</td>
<td>Randomized phase II</td>
<td>Letrozole + Temsirolimus 30 mg versus Letrozole + Temsirolimus 10 mg versus Letrozole</td>
<td>PFS</td>
<td>PFS: 13.2 versus 11.5 versus 11.6 months; ORR: 40% versus 33% versus 45%</td>
<td></td>
</tr>
<tr>
<td>Chow, et al., 2006 [43]</td>
<td>NCT00083993 (Phase III)</td>
<td>992</td>
<td>Randomized, double-blind, placebo-controlled multicenter global trial</td>
<td>Letrozol + Temsirolimus versus Temsirolimus versus Letrozole + Placebo</td>
<td>PFS</td>
<td>PFS: 9.2 versus 9.2 months; ORR: 24% versus 24%</td>
<td></td>
</tr>
<tr>
<td>Bachelot, et al., 2010 [44] and Bourgier, et al., 2011 [45]</td>
<td>TAMRAD (Phase II)</td>
<td>111</td>
<td>Randomized</td>
<td>Tamoxifen + Everolimus versus Tamoxifen</td>
<td>CBR</td>
<td>CBR: 61.1% versus 42.1% (P = 0.045); TTP: 8.6 versus 4.5 months, hazard ratio = 0.54 (P = 0.0021); OS: hazard ratio = 0.45 (P = 0.007); TTP in primary resistance: 5.4 versus 3.8 months, hazard ratio = 0.70 (P = non significant); OS in primary resistance: hazard ratio = 0.73 (P = 0.41); TTP in secondary resistance: 14.8 versus 5.5 m, hazard ratio = 0.46 (P = 0.0087); OS in secondary resistance: hazard ratio = 0.21 (P = 0.002)</td>
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<tr>
<td>Bhattacharyya et al., 2011 [46]</td>
<td>Phase II</td>
<td>400</td>
<td>Randomized</td>
<td>Tamoxifen + Sirolimus versus Tamoxifen</td>
<td>RR and TTP</td>
<td>Group 1: RR: 38.8% versus 4.1% (P = 0.00018); TTP: 11.7 versus 3.3 months, hazard ratio = 0.43 (P = 0.0023); TTP in primary resistance: 7.4 versus 2.2 months, hazard ratio = 0.62 (P = non significant); Group 2: RR: 75.6% versus 32.7% (P = 0.0043); TTP: 16 versus 9 months, hazard ratio = 0.48 (P = 0.0028)</td>
<td></td>
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<tr>
<td>Baselga et al., 2011 [39, 47, 48]</td>
<td>BOLERO-2 (Phase III)</td>
<td>724</td>
<td>Randomized, double blind</td>
<td>Exemestane + Everolimus versus Exemestane</td>
<td>PFS</td>
<td>PFS Local assessment: 7.4 versus 3.2, hazard ratio = 0.44 (P &lt; 1 × 10^{-16}); PFS Central assessment: 11.0 versus 4.1, hazard ratio = 0.36 (P &lt; 1 × 10^{-16})</td>
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</table>

BC, breast cancer; CBR, clinical benefit rate; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; mTOR, mammalian target of rapamycin; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor; RR, response rate; TTP, time to progression.
An antiproliferative response, as defined by a reduction in Ki67 expression, occurred in 57% patients in the everolimus arm versus 30% in the placebo arm ($P < 0.01$). The use of early changes in Ki67 as an intermediate marker of neoadjuvant treatment has been addressed in other studies and has correlated positively with clinical and/or pathological response in early BC with hormone therapy and chemotherapy [40, 41].

This study showed that everolimus increased the efficacy of letrozole in the treatment of newly diagnosed ER-positive BC in terms of both clinical and antiproliferative response.

mTOR inhibitors — advanced BC

temsirolimus. Baselga et al. [42] conducted a phase II study in 92 women that compared the efficacy and safety of daily letrozole alone or in combination with daily temsirolimus (Table 2). Patients in the temsirolimus group had a longer PFS compared with those receiving letrozole alone (18.0 versus 9.5 months, respectively).

Given these results, a phase III, randomized double-blind trial evaluating temsirolimus in combination with letrozole in postmenopausal women with locally advanced or MBC was conducted [43]. Nine hundred and ninety-two women were randomly assigned in a 1:1 ratio to receive oral temsirolimus (30 mg daily for 5 days every 2 weeks) or placebo in combination with letrozole. There were no differences in overall response rates (ORRs), clinical benefit rates (CBRs) and PFS between the two groups at the interim analysis, suggesting that the addition of temsirolimus to letrozole provided no improvement in clinical outcome in postmenopausal women with advanced BC or MBC.

everolimus. TAMRAD phase II trial TAMRAD is a phase II trial that enrolled 111 patients with HR-positive HER2-negative MBC who had previously received adjuvant therapy with an AI [44]. After stratification according to primary or secondary hormone resistance (determined by early or late progression after previous AI treatment), patients were randomly assigned 1:1 to receive either tamoxifen alone or in combination with everolimus (10 mg/day). The primary endpoint of the trial was CBR. In an exploratory analysis, the CBR was 42% for the tamoxifen group (TAM) and 61% ($P = 0.045$) for the tamoxifen/everolimus group (RAD/TAM) [45]. Similarly, TTP favored the combination group (4.5 versus 8.6 months; hazard ratio = 0.54, $P = 0.0021$), as did OS (hazard ratio = 0.45, $P = 0.007$).

CBR differences were particularly increased in patients with secondary hormone resistance (44% for TAM versus 74% for RAD/TAM). Looking at TTP as a function of intrinsic hormone resistance, Bachelot noted that among patients with primary resistance, TTP was 3.8 months for TAM and 5.4 months for the combination (hazard ratio = 0.70, $P = \text{non significant}$). Among those with secondary hormone resistance, TTP was 5.5 months for TAM and 14.8 months for RAD/TAM (hazard ratio = 0.46, $P = 0.0087$). OS was significantly better among patients with secondary resistance (hazard ratio = 0.73, $P = 0.41$ versus hazard ratio = 0.21, $P = 0.002$).

Based on these results, the investigators plan to conduct additional studies evaluating the combination of everolimus
and hormonal therapy as a second-line option for women with HR-positive HER2-negative BC.

**BOLERO-2 phase III trial.** BOLERO-2 is a phase III that enrolled 724 women postmenopausal women with advanced ER-positive HER2-negative BC who were refractory advanced BC (with recurrence or progression following prior therapy with letrozole or anastrozole) [47, 49]. After initial presentation during 2011 European Society of Medical Oncology conference, updated results were reported during San Antonio Breast Cancer Symposium 2011, with a median follow-up of 12.5 months [48]. Patients were randomly allocated in a 2 : 1 ratio to receive everolimus 10 mg daily or placebo, with both arms receiving exemestane. The primary end point for the trial was PFS. No crossover after disease progression was allowed. Previous therapies included tamoxifen, fulvestrant, and one chemotherapy regimen. By protocol definition, 84% of patients had previous sensitivity to hormonal therapy (response or long stabilization in the metastatic setting or at least 2 years of adjuvant therapy).

The trial was stopped early after the February 2011 prespecified interim analysis found a significantly better PFS by local assessment for the combined therapy group: median 7.4 versus 3.2 months (hazard ratio = 0.44, \( P < 1 \times 10^{-16} \)). Based on central assessment, everolimus increased median PFS from 4.1 to 11.0 months (hazard ratio = 0.36, \( P < 1 \times 10^{-16} \)). The consistency of the treatment effect was observed in each of all these prospectively defined subgroups with an estimated hazard ratio ranging from 0.25 to 0.60. Overall RR and CBR were significantly greater in the combination group (12% versus 1%, \( P < 0.0001 \) and 51% versus 26%, \( P < 0.0001 \), respectively). Survival was immature at the time of the interim analysis with a total of 83 deaths: 11% in the combination arm and 13% in the exemestane arm. Although grade 3–4 side-effects were more often in the combination arm, this did not translate into differences in quality of life.

This is the first, large phase III study of a targeted agent, everolimus, which, in combination with endocrine therapy, reported significantly improved PFS, RR, and a manageable safety profile. The trial results were reported earlier than expected at the first interim analysis as the outcome of combination had exceeded the prespecified PFS threshold for significance. As a result, OS data are still immature and are eagerly anticipated.

The discordant results between the temsirolimus and everolimus trials are not well understood. One reason that might explain this is that population was different between both studies: the temsirolimus trial included only endocrine treatment-naïve patients, while the everolimus population was composed of patients refractory to a previous treatment with AI. In addition, the different outcomes seen between studies might be due that temsirolimus was not bioactive enough in the study due to a high rate of toxic effects: grade 3–5 adverse events occurred in 37% versus 11% in the temsirolimus and everolimus groups, respectively [43, 49].

*sirilimus in MBC.* Bhattacharyya et al. [46] recently presented the results of a trial that evaluated the addition of tamoxifen (TAM) to sirolimus (SIR) in HR-positive HER2-negative MBC. The study was done in two groups including 400 patients: (i) prior exposure to AIs or failed on TAM within 6 months and (ii) no prior exposure to AIs. The primary end points were RR and TTP. The results of the group 1 showed RR of 4% versus 39% \( (P = 0.00018) \) and TTP was 3.3 versus 11.7 months (hazard ratio = 0.43, \( P = 0.0023 \)), for TAM and TAM/SIR, respectively. Notably, for those patients who progressed within 6 months, the magnitude of this effect was lower (TTP 2.2 versus 7.4 months, hazard ratio = 0.62, \( P = \) non significant). For group 2, RR was 33% versus 76% \( (P = 0.0043) \) and TTP was 9.0 versus 16.0 months (hazard ratio = 0.48, \( P = 0.0028 \)). The conclusion of this study is that combination treatment increased RR and TTP while showing a greater quality of life adjusted for survival.

**biomarkers**

Two mTOR activation biomarkers were assessed in 35 patients in the primary tumor in the TAMRAD study. pS6K and 4EBP1 are downstream effectors of the mTOR pathway; pS6K is upregulated and 4EBP1 is downregulated by mTOR. Patients with high pS6K expression and low 4EBP1 expression showed the greatest benefit for TTP as a function of biomarker expression. These preliminary results of translational analysis show a possible correlation between biomarkers of mTOR activation and everolimus efficacy.

**mTOR resistance**

Two key regulatory loops have been described that may limit the effectiveness of drugs that have been developed to target mTOR in cancer [32]. The mTOR-activated kinase S6K1 phosphorylates and destabilizes the insulin-receptor substrate 1 and 2 (IRS1 and IRS2) proteins in insulin-like growth factor (IGF)-responsive cells [50]. mTOR inhibition can block the negative feedback on IGF-1R signaling interfering on Akt/PI3K signaling. The result is an increase in Akt phosphorylation, protein kinase activity, and downstream signaling, which could potentially counteract the inhibition of mTOR [31]. Thus, concern has been raised that loss of this negative feedback loop may overcome the antitumor effectiveness of mTOR blockade and limit their effectiveness [51]. Based on preclinical models, dual inhibition of both IGF-1R (with either monoclonal antibodies or tyrosine kinase inhibitors) and mTOR results in a superior antiproliferative effect over each single strategy, and this combination is now under evaluation in phase II trials in patients with BC [31, 52].

In addition, a positive regulatory loop exists involving the mTOR2 complex that is activated directly by growth factors [53]. In contrast to mTOR1, the mTOR2 complex phosphorylates Akt directly, and this is thought to be required for full activation of the Akt pathway by mitogenic signals. As such, mTOR2 complex functions as an upstream regulator of Akt and delivers an additional stimulatory signal to mTOR1. However, rapamycin analogs that target mTOR proteins appear to specifically only block the mTOR1 complex and do not inhibit the mTOR2 complex [54, 55].
toxicity and practical implications

management of toxic effects

The main toxic effects associated with the use of mTOR inhibitors are stomatitis, pneumonitis, and metabolic abnormalities, as shown in Table 3. These toxic effects can influence the practical use of this class of drugs and compliance.

Since temsirolimus [56] and everolimus [57, 58] have been approved for the treatment of metastatic renal cell carcinoma since 2007 and 2008, respectively, most of the experience in the management of toxic effects related to this compounds come from these studies. Recently, an expert group published the guidance for management of selected adverse effects associated with the use of everolimus for the treatment of metastatic renal cell carcinoma [59]. As we integrate these agents in BC, it is critical that clinicians are educated about these key recommendations and specific guideline modifications. Some of the key issues are summarized below:

stomatitis. The efficacy of specific topical corticosteroids and mouthwashes in the treatment of chemotherapy-induced stomatitis has been reviewed extensively and may be applicable to patients treated with everolimus [60, 61]. The main preventive measurements include good oral hygiene, treatment of anticipated infectious foci, and avoidance of alcohol- or peroxide-containing products, as they may exacerbate the condition. In addition, patients should be evaluated for herpes and fungal infections, with institution of an antiviral agent or an antifungal agent as appropriate.

interstitial pneumonitis. In patients with baseline respiratory symptoms or in patients with documented multiple lung metastases, a computed tomography scan and lung function tests, and arterial oxygen saturation should be carried out before everolimus is initiated. A temporary treatment interruption may be considered if symptoms are moderate or severe; following resolution of symptoms, everolimus may be reinitiated at a reduced dosage of 5 mg/day.

The extent to which prior chest radiation increases the risk of mTOR inhibitor-induced noninfectious pneumonitis is unclear. A clinical study of patients with metastatic or recurrent BC showed prominent radiological findings ipsilateral to previous adjuvant radiation fields [62], which suggests that previous radiotherapy may be an influencing factor for developing everolimus-associated pneumonitis.

metabolic abnormalities. Mild metabolic abnormalities can be managed routinely without treatment interruption. Intervention at the grade 2 level is recommended, with the extent of intervention dependent on the specific metabolic abnormality. Patients with underlying diabetes require careful monitoring and, potentially, modifications to their antihyperglycemic regimen. Optimal glycemic control before everolimus initiation is mandated since hyperglycemia primarily occurs in patients with abnormal pretreatment fasting glucose levels.

perspectives

There is an unmet therapeutic need in endocrine-resistant, ER-positive HER2-negative advanced BC. As preclinical studies supported the hypothesis that mTOR inhibition could potentially overcome resistance to endocrine therapy, the evidence from the latest studies support the use of mTOR inhibitors for patients with HR-positive MBC. Based on BOLERO-2 and other supportive results, mTOR inhibition in combination with endocrine therapy will likely be considered a new therapeutic strategy for women with previously AI treated advanced BC.

A better understanding of which patients will most likely benefit from these therapies is essential. Ongoing biomarker analysis and further planned studies integrating biopsies upon initiation of these targeted agents and then on progression are critical to identify predictive markers and select ideal patients likely to benefit.

In addition, in order to overcome the potential development of resistance to mTOR agents, future studies evaluating mTOR inhibition in combination with blockade of compensatory or parallel pathways of mTOR (e.g. IGF-1R inhibitors) will be of particular interest. Along with integration of new agents, there is also need to prospectively study the benefit of continuing the mTOR agents beyond progression.

One important area of study will be the use of a total targeted approach with antiestrogen and mTOR in comparison to chemotherapy in the metastatic setting. One significant impact of prolonging progression-based end points in the metastatic context is the resultant delay in starting chemotherapy for these patients and such a trial end point needs to be considered by investigators as this is greatly valued by patients.

The adjuvant study of mTOR inhibitors is greatly needed based on the results seen in the advanced setting. While HR-positive BC represents more than half of early-stage BC cases, there are only a handful of trials looking at improving the outcome of these patients. Tamoxifen and more recently AIs have significantly improved the outcome of patients with early-stage disease, but still about 15%–20% of these patients will relapse [63, 64]. Careful study of mTOR agents in the early-stage setting is needed in selected patient population. Luminal B tumors, characterized by higher proliferation and more frequently relapses than luminal A cancers, might derive particular benefit from this therapeutic strategy. Importantly, adjuvant trials evaluating these drugs will have to be monitored closely due to potential serious toxic effects such as pneumonitis.

Overall, a significant milestone in the management of HR-positive MBC with the use of mTOR inhibitors has been achieved. After a decade of research, these agents are finally ready for use in the clinical setting. The benefit seen in the pivotal trial BOLERO-2 is clinically meaningful and we need to consider the addition of everolimus to AIs for women with HR-positive MBC. However, a word of caution should be added regarding toxic effects related to these compounds, which might limit practical use and compliance. It is essential that clinicians are educated about key recommendations for toxicity management and specific guideline dose modifications.
Additional research efforts with the addition of these novel compounds in the early-stage setting are greatly needed to improve the survival of patients with HR-positive BC.

acknowledgements
We thank Ivan González-Espinoza, M.D., for his contribution in the development of the tables and figures.

disclosure
CV-G has declared no conflicts of interest. JC is a consultant for Roche, Eisai, Celgene, and Novartis, and is a member of the speakers’ bureau for Roche, Eisai, Cephalon, and Celgene. AF is a member of the speakers’ bureau and advisory board for Novartis, and is currently conducting research sponsored by Novartis. SV is a member of the speakers’ bureau and advisory board for Novartis.

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