Annals of Oncology Advance Access published May 2, 2012

review

*Annals of Oncology*
doi:10.1093/annonc/mds075

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

C. Villarreal-Garza[1], J. Cortes[2], F. Andre[3] & S. Verma[4]*

[1]*Department of Medical Oncology, Instituto Nacional de Cancerologia, Mexico DF, Mexico;* [2]*Breast Cancer Unit, Vall d'Hebron Institute of Oncology, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain;* [3]*Breast Cancer Unit, Department of Medical Oncology, University Paris XI and Institut Gustave Roussy, Villejuif, France;* [4]*Department of Medical Oncology, Sunnybrook Odette Cancer Centre, Toronto, Canada*

*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,

*Correspondence to: Dr S. Verma, Department of Medical Oncology, Sunnybrook Odette Cancer Centre, T-Wing, 2nd Floor, 2075 Bayview Avenue, Toronto, ON M4N 3M5, Canada. Tel: +1-416-480-5248; Fax: +1-416-480-6002; E-mail: Sunil.verma@sunnybrook.ca

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 EFECT 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.

## 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 up regulates 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].

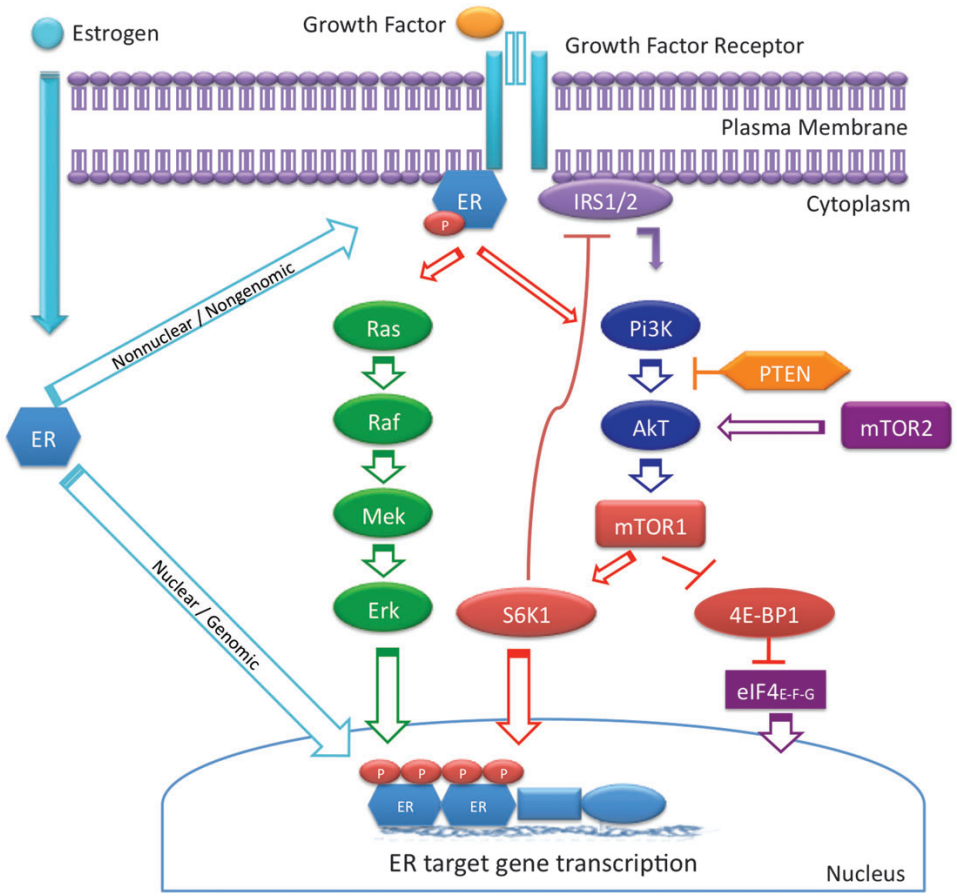


**Figure 1.** PI3K/AkT/mTOR pathway and endocrine genomic and non-genomic cross talk. The PI3K/Akt/mTOR signaling network regulates proliferation, migration, cell survival, metabolism, and apoptosis. This network is dysregulated in BC enhancing translation and cellular proliferation. The mTOR proteins 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.

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 arm. 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, $P$ = 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

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

| Reference | Trial | *n* | Study design | Patients | Treatment | Primary end point | Response/Efficacy |
|---|---|---|---|---|---|---|---|
| Macaskill et al., 2011 [38] | Pilot study | 31 | One arm | Postmenopausal, operable early BC some level of ER+ | Everolimus (5 mg daily) for 14 days before surgery | Percent reduction in *Ki67* in biopsies at diagnosis and surgery | Everolimus resulted in significant reduction in *Ki67* (74%) from baseline ($P = 0.019$) |
| Baselga et al., 2009 [39] | NCT00107016 (phase II) | 270 | Double blind, placebo-controlled, multicenter USA/Europe | Postmenopausal, with previously untreated ER+ BC, eligible for neoadjuvant therapy | Neoadjuvant Letrozole + Everolimus versus Neoadjuvant Letrozole + placebo for 4 months | Response rate by clinical palpation | Response (everolimus versus placebo): CR: 13% versus 9.1%; PR: 55 versus 50%; No change: 25 versus 30%; PD: 4.3 versus 9.8% |

BC, breast cancer; ER, estrogen receptor; mTOR, mammalian target of rapamycin. CR, complete response; PR, progressive disease.

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-naive 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].

*sirolimus in MBC.* Bhattacharyya et al. [46] recently presented the results of a trial that evaluated the addition of tamoxifen (TAM) to sirolumus (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

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.