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REVIEW ARTICLE

# Biomarkers of Everolimus Sensitivity in Hormone Receptor-Positive Breast Cancer

**Zongbi Yi, Fei Ma**

Department of Medical Oncology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Activation of the mammalian target of rapamycin (mTOR) signaling pathway is an important mechanism of resistance to endocrine therapy in breast cancer. Everolimus, an mTOR inhibitor, has been shown to increase the efficacy of endocrine therapy and overcome resistance to endocrine therapies. Clinical studies have suggested that everolimus combined with endocrine therapy prolongs progression-free survival in hormone receptor-positive breast cancer patients. However, because breast cancer includes a group of highly heterogeneous tumors, patients may have different responses to everolimus. Therefore, finding biomarkers that can predict a patient's positive response or resistance to everolimus is critical. Numerous preclinical studies have shown that *PIK3CA/PTEN* mutations are predictive of sensitivity to everolimus; however, clinical trials have not confirmed the correlation between mutation status and clinical response. *KRAS* or *BRAF* mutations can bypass the phosphatidylinositol 3-kinase pathway; therefore, mutations in *KRAS* or *BRAF* may lead to resistance to mTOR inhibitors, and preclinical studies have shown that *PIK3CA* mutant cells which also contain *KRAS* mutations are resistant to everolimus. However, there are no clinical data in breast cancer patients to support this conclusion. Therefore, large-scale clinical studies are needed to identify biomarkers of efficacy and resistance to everolimus.

**Key Words:** *Biomarkers, Breast neoplasms, Everolimus*

## INTRODUCTION

Breast cancer is the most common malignant tumor in women worldwide, thus it is a serious threat to women's health [1,2]. Most breast tumors express hormone receptors (HR), including estrogen receptor and progesterone receptor, and can benefit from endocrine therapy [3]. However, approximately 25% of HR-positive advanced breast cancers show primary or secondary resistance to endocrine therapy [4]. Previous studies have revealed that the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is involved in the mechanisms of resistance to endocrine therapy [5], and mTOR inhibitors have been shown to have the potential to overcome resistance to endocrine therapy [5,6]. As a selective inhibitor of mTOR, everolimus in combination with endocrine therapy could prolong the progression-free survival (PFS) of patients with HR-positive human epidermal growth factor receptor 2 (HER2)-negative breast cancer [7-9]. However, biomarkers that can help select patients who will most benefit from everolimus or those that show resistance to everolimus are urgently needed [10]. This article briefly summarizes the clinical trials of everolimus and reviews the potential biomarkers of everolimus response in HR-positive breast cancer.

**Correspondence to:** **Fei Ma**
Department of Medical Oncology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 17, Panjiayuan Nanli, Chaoyang District, Beijing 100021, China
Tel: +86-10-87787652, Fax: +86-10-87714054
E-mail: drmafei@126.com

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## THE PIK3CA/mTOR PATHWAY IN HORMONE RECEPTOR-POSITIVE BREAST CANCER

mTOR, which acts downstream of the PI3K/AKT pathway, is a serine/threonine protein kinase (Figure 1); as a result of its strategic position, mTOR is an important regulator of many cellular functions [11,12]. mTOR has two major downstream messengers, ribosomal p70 S6 protein kinase 1 (S6K1) and 4E-binding protein (4E-BP1) [11]. Dysregulation of the PI3K/AKT signaling pathway is common in cancer, and this dys-

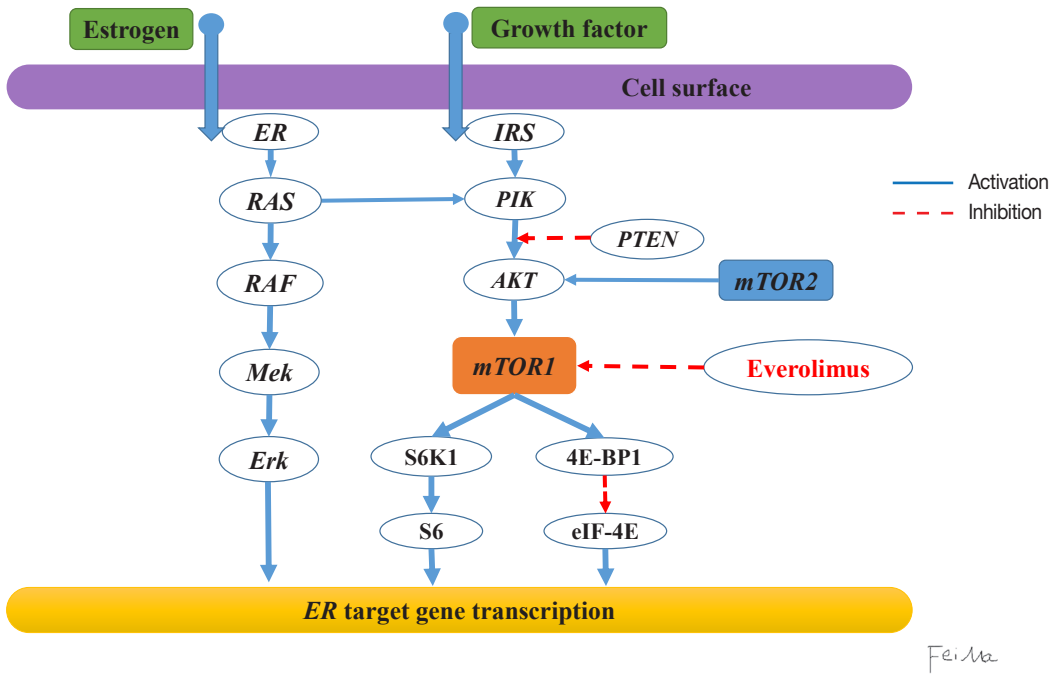


**Figure 1.** The PI3K/AKT/mTOR signaling pathway, showing cascading pathway activation and regulatory feedback loops.
PI3K = phosphatidylinositol 3-kinase; mTOR = mammalian target of rapamycin; ER = estrogen receptor; IRS = insulin-receptor substrate; PTEN = phosphatase and tensin homolog; Erk = extracellular signal-regulated kinase; 4E-BPI = 4E-binding protein 1; S6K1 = ribosomal protein S6 kinase.

regulation can upregulate the mTOR pathway. Alteration of genes in the PIK3CA/AKT pathway is also a frequent occurrence in breast cancer, and the frequency of *PI3K* somatic mutations in breast cancers has been reported to be 20% to 45% [13-17]. Furthermore, phosphatase and tensin homolog (PTEN) can inhibit the activity of the PI3K/AKT pathway, and *PTEN* gene loss has been reported in 15% of breast cancer patients [12]. Numerous studies have shown that tumors can become resistant to endocrine therapy through activation of this pathway. In addition, some preclinical and clinical studies showed that the addition of an mTOR inhibitor to conventional endocrine therapy could restore sensitivity to previously resistant tumor cells and improve disease treatment and the overall survival (OS) of patients with HR-positive breast cancers [18,19].

## CLINICAL STUDIES OF EVEROLIMUS FOR HORMONE RECEPTOR-POSITIVE METASTATIC BREAST CANCERS

### Early-phase clinical trials

Early-phase clinical trials suggested that everolimus may be an optional treatment for HR-positive metastatic breast cancers. A phase I study evaluated the pharmacokinetics and safety of everolimus plus letrozole in patients with metastatic breast cancer. In the study, seven out of 18 patients received combination therapy for more than 6 months. Prominent clinical toxicities were fatigue, stomatitis, diarrhea, anorexia, rash, and headache [20].

Additionally, a phase II study evaluated the safety and efficacy of fulvestrant combined with everolimus for patients with postmenopausal advanced breast cancer that was resistant to aromatase inhibitor. The median time to progression (TTP) in this study was 7.4 months, and the clinical benefit rate (CBR) was 49% [21]. In addition, 71% of the patients in this study received prior chemotherapy, 81% received prior tamoxifen therapy, and 26% received three or more types of endocrine therapy [21]. The most common adverse reactions were mucositis, weight loss, and rash. This study demonstrated that everolimus combined with fulvestrant is effective after aromatase inhibitor resistance in patients with heavily pretreated HR-positive breast cancer, and the toxicities were manageable [21].

### TAMRAD study

The TAMRAD study is a randomized phase II study [9] on patients with HR-positive, HER2-negative metastatic breast cancer who were treated with prior aromatase inhibitor therapy. The purpose of the study was to evaluate the efficacy and safety of everolimus plus tamoxifen compared to tamoxifen


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alone. The results showed CBRs of 61% and 41% for the combination arm and the tamoxifen monotherapy arm, respectively, and this difference was statistically significant ($p=0.04$). The TTP was 4.5 months in patients treated with tamoxifen alone and 8.6 months in patients treated with everolimus plus tamoxifen. The prominent clinical toxicities reported in the combination arm were stomatitis, fatigue, rash, anorexia, and diarrhea. There was no difference in grade 3 or 4 adverse events between the two groups. Subgroup analysis of primary and secondary hormone resistance indicated that the median TTP was 14.8 months in patients with secondary resistance versus 5.4 months for patients with primary resistance. Similarly, patients with secondary resistance to aromatase inhibitors had a significantly higher CBR when treated with everolimus combined with tamoxifen (74%) than those treated with tamoxifen alone (48%). This study revealed that combination therapy with tamoxifen and everolimus increased the TTP, CBR, and OS when compared to tamoxifen monotherapy in patients with aromatase inhibitor-resistant postmenopausal metastatic breast cancer [9].

### BOLERO-2 study

The promising results observed in the phase II studies of everolimus warranted further studies in patients with HR-positive, HER2-negative metastatic breast cancers, especially patients with *de novo* resistance to aromatase inhibitors. The BOLERO-2 study is a phase III study that compared everolimus combined with exemestane to placebo plus exemestane for patients with HR-positive HER2-negative breast cancer that is resistant to nonsteroidal aromatase inhibitor therapy. The results from the final analysis, after a median follow-up of 18 months, indicated that the primary end-point median PFS was 7.8 months in the everolimus plus exemestane arm versus 3.2 months in the placebo plus exemestane arm ($p=0.0001$). The CBR in the everolimus arm was significantly higher than that in the placebo arm (51.3% vs. 26.4%, $p=0.0001$) [7]. The median OS was 31.0 in patients treated with everolimus plus exemestane and 26.6 months in those treated with placebo plus exemestane; however, this difference was not statistically significant ($p=0.1426$) [8].

## BIOMARKERS OF EFFICACY

### *PIK3CA* gene mutations

Tumors harboring mutations in genes encoding proteins involved in the PI3K/AKT/mTOR pathway may activate the PI3K enzyme. Therefore, such tumors are expected to be sensitive to everolimus and agents targeting this pathway. The results of several preclinical studies have suggested that genetic aberrations in the PI3K/AKT/mTOR pathway could predict the efficacy of mTOR inhibitors [22,23]. However, data from clinical studies regarding the predictive capability of these genetic aberrations are contradictory. A retrospective study showed that patients with advanced breast cancer treated with inhibitors of the PI3K/AKT/mTOR pathway in combination with endocrine therapy, anti-HER2 therapy, or chemotherapy had a longer TTP compared to patients with wild-type tumors [24]. Baselga et al. [25] analyzed the relationship between the presence of mutations in exon 9 of *PIK3CA* and the efficacy of everolimus plus letrozole in a neoadjuvant trial. The results from the study indicated that the presence of these *PIK3CA* mutations provided an improved response to the combination of everolimus with letrozole [25]. However, in the TAMRAD trial, the researchers analyzed the relationship between mutations in the primary tumor tissue and the efficacy of everolimus, and they did not find any correlation between the presence of *PI3K* mutations and the response to everolimus [26]. Although they did find that everolimus was more effective in patients with low *PI3K* expression. In addition, patients with low levels of liver kinase B1 (LKB1), a known suppressor of mTOR, and high levels of phospho-4E binding protein, which is downstream of mTOR, received a greater benefit from everolimus treatment [26]. The BOLERO-2 study also failed to identify any specific gene mutations in the tumor tissue that were associated with a greater benefit from everolimus treatment [27]. Nevertheless, the investigators found that patients with no alteration or a single genetic alteration in *PIK3CA/PTEN/CCND1* or *FGFR1/2* received a greater PFS benefit from everolimus treatment [28]. In the BOLERO-2 study, mutation analysis of plasma cell-free DNA (cfDNA) suggested that there was no relationship between the PFS of patients treated with everolimus and the *PIK3CA* genotypes in cfDNA, which was consistent with previous tumor tissue DNA analysis. These results suggest that *PIK3CA* mutations, including *H1047R*, *E545K*, and *E542K*, cannot predict patient response to everolimus. In conclusion, the current evidence is insufficient to demonstrate that the *PIK3CA* genotype is an effective predictive biomarker for everolimus benefit [29].

### *PTEN* gene mutations

Some *PTEN* gene variations, including germline and somatic mutations, can activate the PI3K enzyme. Preclinical models support the notion that cells with *PTEN* gene loss are more sensitive to PI3K/AKT inhibitors [30,31]. However, the results from the TAMRAD trial showed that *PTEN* gene loss did not influence the response to everolimus [26]. In a similar analysis in the BOLERO-2 trial, *PTEN* gene status was not correlated with clinical outcome, which seems to confirm the

results reported by the TAMRAD study [27].

### Other gene biomarkers

In one study, a panel of breast cancer cell lines with *HER2* amplification was sensitive to everolimus [32]. Another study showed that the combined presence of *HER2* gene amplification along with *PIK3CA* mutation was highly predictive of sensitivity to an inhibitor of the PI3K/AKT/mTOR pathway (GDC-0941) [31]. Preclinical studies showed that the mTOR pathway is regulated by neurofibromin, a protein encoded by the *NF1* gene, and several other studies demonstrated that cells containing mutations in the *NF1* gene were highly sensitive to mTOR inhibitors, including everolimus and rapamycin [33]. A recent study explored the genomic alterations that confer extreme sensitivity to everolimus in 39 tumors of various types from patients who were treated with everolimus. The results showed that patients with *mTOR*, *NF1*, *PIK3CA*, *TSC1*, *TSC2*, and *PIK3CG* mutations could benefit from the mTOR inhibitor everolimus. Conversely, *BAP1* and *FGFR4* mutations were noted only in patients who did not receive a clinical benefit from everolimus. However, there were no breast cancer patients enrolled in this study [34]. In the BOLERO-2 study, researchers explored the correlation between *ESR1* mutations, including *Y537S* and *D538G*, in cfDNA and sensitivity to exemestane and everolimus. Interestingly, these two activating mutations of the *ESR1* gene appeared to have differential effects on sensitivity to everolimus. The results indicated that patients with the *Y537S* or *D538G* mutations had a poorer prognosis and shorter OS; however, the PFS of patients treated with everolimus was lower in those with the *Y537S* mutation than in those with wild-type *ESR1*, whereas the magnitudes of the PFS benefit of everolimus in patients with wild-type *ESR1* and those with the *D538G* mutation were similar [35].

### Protein biomarkers

Preclinical studies in tumor cell lines indicated that high levels of phosphorylated AKT, glycogen synthase kinase 3 β, and tuberous sclerosis complex 2 are correlated with increased sensitivity to everolimus [36]. O'Reilly et al. [37] investigated potential predictive protein biomarkers for sensitivity to everolimus in human and animal studies, and univariate analysis showed that the levels of pS6, total S6, pS6/total S6, and phospho-AKT (pAKT) were significantly correlated with sensitivity to everolimus. Further analysis found that the combination of high pAKT and high p235-S6/total S6 levels could be a predictor of sensitivity to everolimus, whereas low pAKT and low p235-S6/total S6 levels could be a predictor of insensitivity to everolimus [37]. The TAMRAD trial found that everolimus was more effective in patients who had low PI3K expression, low LKB1 expression, and high phospho-4E binding protein expression; however, they did not find a relationship between the presence of *PI3K*, *PTEN*, and *pAKT* mutations and the efficacy of everolimus [26]. Baselga et al. [25] evaluated tumor core biopsies before treatment and on day 15 of treatment in patients treated with letrozole and either everolimus or placebo in a neoadjuvant trial of everolimus. The results indicated that patients treated with everolimus had a statistically significant decrease in Ki-67 and pS6 levels [25].

## BIOMARKERS OF RESISTANCE

Clinical studies of PI3K/AKT/mTOR pathway inhibitors indicate that a larger number of patients develop *de novo* resistance to this class of anticancer agents. Therefore, understanding the biological basis of this *de novo* resistance and identifying biomarkers of resistance to this therapy are very important. *KRAS* or *BRAF* mutant proteins can bypass the PI3K/AKT/mTOR pathway. Therefore, patients with *KRAS* or *BRAF* mutations may show resistance to PI3K/AKT/mTOR inhibitors. Indeed, preclinical studies suggest that cells containing *KRAS* mutations are insensitive to everolimus [38]. Interestingly, a study of an non-small cell lung cancer (NSCLC) cell line suggested that *de novo* resistance to everolimus mediated by *KRAS* mutation is mitigated by a coexisting LKB1-deficiency as well as p53 loss. Importantly, these data suggest that *LKB1/KRAS*-mutant NSCLCs might be sensitive to mTOR-targeted therapies. Mahoney et al. [39] investigated the correlations between *KRAS* or *BRAF* mutation status and the efficacy of a *PIK3CA* inhibitor in a phase I clinical trial. The results indicated that colorectal cancer patients with *PIK3CA* or *KRAS* mutations did not respond to therapy, whereas patients with *PIK3CA* mutant ovarian cancer that also carried a *KRAS* or *BRAF* mutation did respond to *PIK3CA* inhibitor therapy. This study supported the hypothesis that the effects of *BRAF* and *KRAS* mutations may differ among tumors. However, there is no evidence for a relationship between *KRAS* or *BRAF* mutation status and the efficacy of everolimus in breast cancer.

Acquired resistance to everolimus may also be mediated by genetic alterations generated under the selective pressure of this agent. Although no such genetic alterations were reported in human trials, Zunder et al. [40] performed a preclinical study in *Saccharomyces cerevisiae* and identified a mutational hotspot in the Ile800 area of the *PIK3CA* gene, which confers a 5- to 10-fold decrease in potency for a large panel of PI3K and mTOR inhibitors. Unlike tyrosine kinase inhibitors, these resistance mutations do not reside in the classic gatekeeper residues [40], and cfDNA analyses in the BOLERO-2 study


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showed that the *ESR1* mutation *Y537S* may be a resistance biomarker of sensitivity to everolimus [35].

## CONCLUSION

In summary, activation of the mTOR signaling pathway is an important mechanism of endocrine therapy resistance in breast cancer. As an mTOR inhibitor, everolimus has been shown to increase the efficacy of endocrine therapies and may overcome drug resistance in HR-positive metastatic breast cancer. Mutations in the PIK3CA/AKT/mTOR pathway and *PTEN* gene loss are frequently observed in breast cancer patients, and numerous preclinical studies have demonstrated that mutations in *PIK3CA* could be predictive biomarkers of everolimus efficacy [22,23]. However, the clinical trials have not supported this. None of the clinical studies have found any association between *PIK3CA* mutation status and clinical response to everolimus. The results of cell line studies assessing the predictive value of *PTEN* gene loss for everolimus sensitivity have not been consistent, and the clinical studies also did not find any correlation between *PTEN* gene loss and everolimus efficacy. However, this may be due to the heterogeneity of the tumors and the small number of patients included in the studies. It is also possible that the discordance mutational status between primary tumors and metastatic sites influenced the results. Circulating tumor DNA could be a promising biomarker given its potential to overcome the complications associated with tumor heterogeneity. Preclinical studies indicate that *PIK3CA* mutant cells carrying *KRAS* mutations are resistant to everolimus. However, there are no clinical data in breast cancer patients to support this finding. Large-scale clinical studies are also needed to identify biomarkers for sensitivity and resistance to everolimus.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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