

## The Role of Mtor Inhibitors in Endocrine Resistance Therapy of Breast Cancer Type ER+

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### Abstract

Breast cancer is the leading cause of death in women. Although hormonal therapy has produced positive results in HR+ breast cancer, resistance to this therapy remains a challenge in the treatment of breast cancer. Various mechanisms have been proposed as an explanation of resistance in breast cancer, one of which is the activation of the PI3K/AKT/mTOR pathway. The PI3K/AKT/mTOR pathway is a transduction pathway that regulates many important aspects in both normal cells and cancer cells, including cell proliferation, apoptosis, cell morphology and migration, protein synthesis, and integrase metabolism. Preclinical dan clinical data show inhibition of this pathway can improve survival, especially in resistant hormonal therapy models. The use of mTOR inhibitors (everolimus) has been approved by the FDA as a combination therapy with exemestane for postmenopausal women with HR+/HER2-breast cancer.

**Keywords:** breast cancer, hormonal therapy resistance, PI3K/AKT/mTOR, mTOR inhibitors

### 1. Introduction

Breast cancer is the second cause of tumor death-related in Asia.<sup>1</sup> Breast cancer is classified by the expression of estrogen receptors (Estrogen Receptor – ER), progesterone receptors (Progesterone Receptor - PR), and human epidermal growth factor receptor 2 (HER2).<sup>2</sup> Approximately 70% of human breast cancer is a breast cancer subtype ER+. This subtype needs estrogen for growth and survival.<sup>3,4</sup>

The standard treatment for this type is endocrine therapy or hormonal therapy by inhibiting the transduction of estrogen signals or lowering estrogen levels. The endocrine therapy used nowadays are selective estrogen receptor modulators (SERMs, e.g. tamoxifen), selective estrogen receptor down-regulators (SERDs, e.g. fulvestrant), and aromatase inhibitors (such as anastrozole, letrozole, and exemestane).<sup>5</sup>

However, 50% of ER+ breast cancer patients do not respond (refractory) or stop

responding (resistance) to this endocrine therapeutic agent.<sup>1,4,6</sup>

Mechanisms involved in resistance are modification of ER expression, increased expression of co-activators, variations in the expression of CYP450, and increased transduction of growth factor signals. Upregulation of growth factor receptor pathways, especially HER2 receptors, EGF, and IGF is a major cause of breast cancer. The main signal transduction pathway for such growth factor receptors is PI3K/AKT/mTOR. mTOR modulation can be one of the strategies to overcome resistance to endocrine therapy.<sup>6-8</sup>

### 2. Breast Cancer Therapy Type ER +

Various types of breast cancer therapy are SERMs, SERDs, and aromatase inhibitors.<sup>3</sup>

#### 2.1. SERMs (*selective estrogen receptor modulators*)

Over the past decades, selective ER modulators (tamoxifen) have been used widely as a treatment for breast cancer. Tamoxifen

has a selective estrogenic effect (on the uterus, liver, and bones) and antiestrogens (on the breast and brain). When tamoxifen binds to LBD (ligand binding domain) in ER $\alpha$  in the breast epithelium, the drug induces a conformation that recruits the co-repressor thereby inhibiting estrogen from binding to its receptors and preventing the proliferation of the ER $\alpha$  signal transduction pathway.<sup>8</sup> However, tamoxifen has an unwanted effect due to its estrogenic activity on other tissues such as vaginal bleeding, endometrial hyperplasia, and endometrial cancer.<sup>4,7,10</sup>

Tamoxifen is a prodrug and metabolized in the liver by CYP2D6 and CYP3A4. Raloxifene is another class of nonsteroidal SERMs incorporated in benzothiophene, this agent can produce less toxicity than tamoxifen.<sup>10,11</sup>

### 2.1. SERDs (*selective estrogen receptor down-regulators*)

SERDS (example fulvestrant) acts as ER antagonists without specific agonist traits. These drugs through ER $\alpha$  binding to LBD make conformations that do not correspond to transcription activity. This drug has a second important effect on ER $\alpha$  by targeting receptors for proteasome degradation. The main drawback of this agent is its low oral bioavailability requiring intramuscular administration of the drug. A new generation

of SERD group agents developed to address these deficiencies, such as GDC0810.<sup>7,12</sup>

### 3. Mechanism of Hormonal Therapy Drugs Resistance

Resistance in cancer cells can be intrinsic or acquired. Several resistance mechanisms of breast cancer are loss of ER $\alpha$  expression, ER genome (ESR1) or epigenetic aberrations, dysregulation of ER $\alpha$  and ER $\beta$  isoform expression, post-translational modification of ER $\alpha$ , increased AP1 activity and growth factor signals as well as deregulation of ER coregulators, cell cycles, and apoptosis.<sup>3,5</sup>

Expression amplification on growth factor receptors such as FGFR1, HER2, HER3, EGFR, and IGF1R causes excessive activation in the PI3K-AKT-mTOR pathway. It is associated with the resistance of breast cancer cells to endocrine therapy. Excessive activation of these pathways increases proliferation and survival defenses so that breast cancer cells can avoid endocrine therapy.<sup>3,5,13,14</sup>

ER activation from PI3K-AKT pathways is not dependent on ligands because this pathway promotes ER phosphorylation at certain serine residues. In addition, cross-talk between PI3K-Akt-mTOR and Ras-Raf-MEK-MAPK can improve the proliferation and life-defense capabilities of malignant cells leading to resistance in endocrine therapy.<sup>14</sup>

**Table 1. Clinical trials using mTOR inhibitors in ER+ breast cancer that have been receiving therapy<sup>21-26</sup>**

Study	Therapy	Population	Predecessor Therapy	Result
<b>BOLERO-2</b>  Breast Cancer Trials of Oral Everolimus-2	Everolimus + Exemestane vs Placebo + Exemestane	Breast cancer HR+, HER2- N=724	Anastrozole/letrozole	Progression Free Survival (PFS) 11 months EVE+EXE vs 4,1 months PBO+EXE (95% CI, p < 0,0001)
<b>TAMRAD</b>  Tamoxifen Plus Everolimus (Rad)	Everolimus + tamoxifen vs tamoxifen	Breast Cancer HR +, HER2- N = 111	Aromatase Inhibitor	CBR 61 % vs 42 % (p=0,045) PFS 8,6 vs 4,5 months (p=0,02)
<b>BOLERO-3</b>	Everolimus + vinorelbine + trastuzumab vs placebo + vinorelbine + trastuzumab	Breast cancer HER2+ Resistant N = 569	taxane + trastuzumab	PFS 7 vs 5,78 months (95% CI, p=0,0067)

#### 4. PI3K/AKT/MTOR Pathway

Preclinical studies show that PI3K/AKT/mTOR activation causes acquired cancer cell resistance mechanisms to hormonal therapy, including tamoxifen, aromatase inhibitors, and fulvestrant. The distorted PI3K intracellular signals occur in approximately 70% of breast cancers, including PIK3CA mutations (found 30% in ER+ breast cancer); mutations or amplifications in other genes encoding PI3K subunits, mutations in downstream effectors AKT1, AKT2, PDK1, and loss of inhibitory signals from lipid phosphatase PTEN and INPP4B.<sup>7,12,13,15-17</sup>

mTOR (mammalian target of rapamycin) complex is a serine/threonine kinase protein that regulates proliferation, motility, cell growth, life defense and apoptosis, protein synthesis, and metabolism. The complex consists of two multiprotein complexes mTORC1 and mTORC2. mTORC1 regulate translational and protein synthesis through p70 ribosomal S6 kinase (p70S6K) and eukaryotic initiation factor 4E binding-protein-1 (4E-BP1). After phosphorylation, 4E-BP1 will dissociate from the cap-binding mRNA eIF4E protein. 4E-BP1 then forms a translational initiation complex with eIF4G. P70S6K can phosphorylate ERαserine 167 which can increase ligand-dependent transcription activity and ligand-dependent transclusion activity. Inhibition of mTORC1 will also inhibit ER1 domain. The mTORC2 complex is involved in cell proliferation through glucocorticoid activation of protein kinase 1 (SGK1) and direct activation of Akt. SGK1 promotes resistance to chemotherapy and Akt inhibitors.<sup>7,12,13,15-18</sup>

#### 5. mTOR Inhibitor

mTOR inhibitors act by inhibiting 4EBP1 thus will reduce the translation of cyclin D1 and eliminate CDK inhibitors (p27). In addition, it can also inhibit the transition from the G1-S and polymerase I-III. Evidence shows that inhibition of mTOR can increase letrozole

activity in MCF-7 aro cells and ER1 breast cancer cells. In addition, the administration of pan-AKT-MK-2206 and mTOR rapamycin combined with anastrozole can restore its sensitivity to endocrine therapy.<sup>4,15,19,20</sup>

mTOR inhibitors approved by the FDA include:

##### 1. Rapamycin (known as sirolimus)

Rapamycin, the first class of mTOR inhibitors, selectively inhibits mTOR by forming a complex with intracellular immunophilin FKBP12. At low concentrations, rapamycin suppresses the phosphorylation of the S6K protein, thereby stopping cell cycle. Whereas at higher concentrations, rapamycin can induce apoptosis by causing complete dissociation of Raptor mTORC1, thus suppressing the phosphorylation of 4EBP1.<sup>7,18</sup>

##### 2. Temsirolimus

In May 2017, temsirolimus was approved by FDA for renal cell carcinoma (RCC). Temsirolimus is a prodrug that will be converted to rapamycin. mTOR administration was best given in patients with acquired or secondary resistance. HORIZON study phase III showed administration emsirolmus with letrozole or letrozole/placebo gave meaningless results. However, this trial was not conducted on patients who had received previous hormonal therapy.<sup>7,8</sup>

##### 3. Everolimus

Everolimus is an allosteric mTOR antagonist by binding FKBP12 to the rapamycin-FKBP12 binding domain of mTOR. It does not affect TORC2, so inhibition of MTORC1 can cause negative feedback activity thereby increasing the phosphorylation of AKT from TORC2. New inhibitors is being develop that can inhibit TORC1 and TORC2 such as AZD2014 or MLN0128. FDA has approved exemestane-everolimus for postmenopausal women with HR+/HER2-. Everolimus recommended dosage is 10 mg peroral per day combined with exemestane 25 mg peroral per day. If toxicity levels in level 2/3, dose

reduction should be done with an initial decrease up to 5 mg per day followed by a subsequent decrease up to 5 mg the next day.<sup>7,8,18</sup>

#### 4. Ridaforolimus

Ridaforolimus has been approved by FDA for bone sarcoma with soft tissues metastases.<sup>7</sup>

### 6. Conclusion

Although endocrine therapy is the main therapy in the treatment of ER+ breast cancer, de resistance to these drugs have arises in many patients. These encourages the need for new treatments. Preclinical and clinical evidence suggests PI3K/Akt/mTOR pathways play important role in the development and proliferation of breast cancer. Everolimus is the only mTOR inhibitor currently approved by the FDA. The BOLERO-2 studies proved mTOR can increase PFS. However, everolimus toxicity can affect patient quality of life. Another approach being developed is to use mTORC1/mTORC2 inhibitors or PI3K/mTOR inhibitors which are expected to have better efficacy and safety.

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