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Review Article

Everolimus: a new hope for patients with breast cancer

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Abstract

Background: Breast cancer cells can develop resistance to standard hormonal treatment and chemotherapy with the activation of mTOR pathway which supported by resulted preclinical and clinical studies. In resulted clinical trials, addition of everolimus to hormonal treatment or anti-HER2 treatment improved the outcomes of breast cancer patients. The aim of this review is to discuss the efficacy and safety data of everolimus in all categories of breast cancer in the era of published recent studies.

Scope: Everolimus showed positive results in clinical studies. Literature search is made from PubMed, ASCO and San Antonio Breast Cancer Symposium Meeting abstracts by using the following search keywords; “everolimus”, “RAD001”, “mTOR inhibitor”, “breast cancer” “endocrine therapy resistance” and “HER-2 targeted therapies”. The last search was on June 10, 2013. The most important limitation of our review is that the most of the data of everolimus reliance on to phase I and II trials.

Findings: Preclinical studies showed that mTOR activation can be the responsible mechanism in all subgroups of the breast cancer. Results of both TAMRAD and BOLERO-2 studies have showed that mTOR inhibition in combination with endocrine therapy can be a new treatment strategy for MBC patients who resistant to aromatase inhibitors. In BOLERO-2 study, time to deterioration in health-related quality of life was also significantly higher in everolimus and exemestane arm compared to exemestane plus placebo arm. Recently completed BOLERO-3 study showed that mTOR inhibition in combination with trastuzumab plus vinorelbine treatment significantly improved PFS compared to trastuzumab plus vinorelbine alone in trastuzumab-resistant MBC patients.

Conclusion: Recent trials showed that everolimus has produced promising anti-tumor activity in combination with trastuzumab in HER2-positive metastatic breast cancer and in combination with exemestane in hormone receptor-positive metastatic breast cancer who had recurrence or progression while receiving nonsteroidal aromatase inhibitor. With the results of ongoing studies with everolimus in the future may tending towards of these agents in earlier stages of the disease, namely in the adjuvant and neoadjuvant settings.

Review Article

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ABSTRACT

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Conclusion: Recent trials showed that everolimus has produced promising anti-tumor activity in combination with trastuzumab in HER2-positive metastatic breast cancer and in combination with exemestane in hormone receptor-positive metastatic breast cancer who had recurrence or progression while receiving nonsteroidal aromatase inhibitor. With the results of ongoing studies with everolimus in the future may tending towards of these agents in earlier stages of the disease, namely in the adjuvant and neoadjuvant settings.

Keywords: Everolimus; breast cancer; mTOR pathway; trastuzumab resistance; endocrine therapy resistance

Introduction

Breast cancer is the most commonly diagnosed cancer type in women and expected to account for 29% of all new cancer cases among women in 2013 with stable incidence rate from 2005 [1,2]. Although death rates of breast cancer have decreased by more than 30% over the past two decades, breast cancer is still the second leading cause of death after lung cancer due to cancer deaths among women all over the world [3]. With the improvements in screening and treatment modalities, mortality of breast cancer appears to be declining [4,5].

Despite overall survival (OS) of breast cancer increases in recent years, lack of response in some patients and relapse during the course of therapy continue to challenge researchers and clinicians towards a better understanding of the fundamental mechanisms of breast cancer. Thus, there is a clear need for the development of new agents targeting of dysregulated cell signaling pathways in breast cancer [6,7].

Mammalian target of rapamycin (mTOR) kinase pathway plays an important role in cell survival, proliferation, angiogenesis and metabolism [8,9]. mTOR, a serine/threonine protein kinase that belongs to the phosphatidylyinositol 3-kinase (PI3K) protein family, is frequently activated during carcinogenesis via genetic and epigenetic alterations. Thus mTOR pathway contributes to the development and progression of breast cancer [8]. Activation of mTOR pathway leads to PI3K gene activation, phosphatase and tensin homolog (PTEN) loss and high levels of AKT expression in 16-48% of breast cancer patients [10-12]. High levels of phosphorylated mTOR was correlated with nodal metastatis and poor prognosis in breast cancer patients [13].

mTOR pathway overactivated approximately in all breast cancer subgroups. The HER family, estrogen receptor (ER) family and insulin-like growth factor receptor family are all related with the activation of mTOR pathway [14]. Preclinical studies demonstrated that

PI3K/Akt/mTOR pathway was found to be associated with the response of hormonal treatment, chemotherapy and targeting agents in breast cancer cell lines [15,16]. Thus, breast cancer cells can develop resistance to standard hormonal treatment and chemotherapy with the activation of mTOR pathway. Synergistic interactions of mTOR inhibition were observed in combinations with paclitaxel, carboplatin, and vinorelbine in vitro studies. Also additive effects of mTOR inhibition were observed in combinations with doxorubicin and gemcitabine [17].

Everolimus

Everolimus (RAD001) is an oral inhibitor of serine-threonine kinase mTOR that inhibits cell growth, angiogenesis and survival. It inhibits mTOR by binding to its intracellular receptor FKP12 [18]. Everolimus inhibits mTOR through allosteric binding to the mTOR complex 1 (mTORC1) which phosphorylates the activation of the functional domain of ER. Pharmacodynamic studies suggested that 10 mg daily dose is the standard dose of everolimus in cancer patients. The terminal half life of everolimus is 30 hours (26-38 hours) [18]. Everolimus has been approved for the treatment of tyrosine kinase refractory renal cell carcinoma and advanced pancreatic neuroendocrine tumors [19,20]. Also, same biologic activity of everolimus was reported in gastric cancer, lymphoma, hepatocellular cancer and giant cell astrocytoma associated with tuberous sclerosis patients [21,22]. Recently, everolimus was approved in combination with exemestane by the Food and Drug Administration (FDA) for patients with hormone-receptor positive metastatic breast cancer (MBC) patients after failure of nonsteroidal aromatase inhibitor therapy which resulted of the BOLERO-2 trial [23].

HER2 pathway and the role of mTOR inhibitors

Approximately 15-25% of all breast cancers are human epidermal growth factor receptor 2 (HER2) positive and display gene amplification or HER2 overexpression [24-26]. Although the

majority of patients with MBC who initially respond to trastuzumab demonstrate disease progression within 1 year of trastuzumab treatment [27]. Only 30% of HER2-positive breast cancer patients respond to trastuzumab monotherapy and the remaining majority of patients can develop trastuzumab resistance. In vitro studies showed that mTOR activation was related with HER2 overexpression and mTOR pathway was responsible for the HER2-positive breast cancer progression. It was shown that Akt/mTOR hyperactivation was the responsible mechanism of trastuzumab resistance [28]. Another preclinical study showed that mTOR inhibitors can enhance the efficacy of trastuzumab in a xenografts [29]. In addition, HER2 overexpression was associated with carboplatin and paclitaxel resistance in breast cancer cells. In vitro studies, mTOR inhibition enhanced the chemosensitivity of paclitaxel and carboplatin combination in HER2/neu-overexpressing cells, suggesting a potential approach to these poorly behaving tumors [17,30]. On these grounds, mTOR inhibition can be an effective treatment for HER2-positive breast cancer.

Endocrine treatment and the role of mTOR inhibitors

Despite the benefits of endocrine treatment were shown in clinical trials, some of hormone receptor-positive breast cancer patients do not respond to the endocrine treatment and endocrine manipulations. Primary and acquired resistance to endocrine therapy in breast cancer restricts the efficacy of these agents. mTOR pathway induces phosphorylation of ER and resistance to endocrine therapy. Preclinical studies showed that mTOR activation and Akt signaling upregulation was one of the responsible mechanism of endocrine treatment resistance and mTOR inhibitors may contribute to break resistance [31,32]. In vitro studies showed that increasing activity of Akt/mTOR pathway can be the responsible mechanism of letrozole and fulvestrant treatment resistance, thus cotreatment with everolimus restores sensivity of hormonal treatment [32]. In

preclinical studies, the mTOR inhibitor, rapamycin reversed acquired endocrine resistance and inhibited proliferation of ER-positive breast cancer cells at the cell proliferation or gene-expression levels [33,34]. In vitro study of ER-positive breast cancer, addition of everolimus to letrozole reduced breast cancer cell growth and increased the antitumor efficacy of letrozole [35]. Preclinical study in patient-derived xenograft models of endocrine resistant luminal breast cancers targeting the PI3K/mTOR pathway was recently reported. In this study, activation of the PI3K pathway was confirmed in endocrine treatment resistant models and everolimus alone or in combination with endocrine treatment (tamoxifen, letrozole, fulvestrant) confirmed high and durable efficacy [36]. Preclinical datas demonstrated that activation of estrogen independent growth signaling pathways drive resistance to endocrine treatment; thus targeting this pathway can be an effective strategy to overcoming resistance. Based on this rationale, mTOR inhibitors can reverse the resistance to endocrine therapy.

The role of mTOR pathway in triple-negative breast cancer

Triple-negative breast cancers have relatively poor prognosis and can not be treated with targeting agents and hormonal treatment [37]. Due to the lacking of novel therapeutic treatment options in triple-negative breast cancer, finding new targets is very important. Phosphatase and tensin homolog (PTEN) loss was also reported in triple-negative breast cancer patients [38,39]. İt is well known that PTEN loss or dysfunction leads to activation of PI3K/Akt/mTOR pathway; thus due to the mTOR activation, mTOR inhibition can also be a new treatment option in triple-negative breast cancer. Both in vivo and in vitro studies, antitumor activity was reported with everolimus in triple-negative xenograft models [40]. In this study, epidermal growth factor receptor and CK5/6 positivity were found as a predictor markers for response to everolimus in triple-

negative breast cancer. But still yet, no clinical trial has been resulted to show activity of everolimus in triple-negative breast cancer patients.

Breast cancer cells can develop resistance to standard hormonal treatment and chemotherapy with activation of mTOR pathway which supported by resulted preclinical and clinical studies. In resulted clinical trials addition of everolimus to hormonal treatment or anti-HER2 treatment improved the outcomes of breast cancer. The aim of this review is to discuss the efficacy and safety data from the recent studies of everolimus in all categories of breast cancer.

Methods

Publication Search

A computerized search was performed through the Pubmed database, the online abstracts of American Society of Oncology (ASCO) meetings and San Antonio Breast Cancer Symposiums (SABCS) by using the following search keywords; "everolimus", "RAD001", "mTOR inhibitor", "breast cancer" "endocrine therapy resistance" and "HER-2 targeted therapies". The last search was on June 10, 2013. All resulted studies were retrieved and related cited publications also checked for related publications.

Eligible studies

Clinical trials in this review fulfilled all of the following criteria: inclusion of sufficient data to allow estimation of efficacy and safety of everolimus. The language of the published clinical trials were restricted to English.

Phase I trials of everolimus in breast cancer

In a phase I study, addition of everolimus to letrozole treatment, who had received first line or second line endocrine therapy for postmenopausal hormone receptor-positive advanced breast cancer, showed that 38.9% of patients had response more than 6 months with combination

treatment [41]. In this study, symptomatic patients and bulky metastatic patients who need urgent chemotherapy was excluded. Only in one patient (5.6%) complete response (CR) was reported and the most common adverse events were stomatitis (50%), fatigue (44.4%) and decreased of appetite (44.4%).

In a singe arm phase I study, addition of everolimus to paclitaxel and trastuzumab combination showed that overall response rate (ORR) was 44% and median progression free survival (PFS) was 34 weeks in patients with HER2-positive MBC who pretreated with trastuzumab [42]. In this study, 93.9% of patients were pretreated with taxanes and 97.0% of patients were resistant to trastuzumab. Neutropenia was the most common hematological adverse event; grade 3 or 4 neutropenia was reported in 52% of patients. Grade 2 stomatitis was observed in 60.6% of patients, whereas grade 3 stomatitis was observed only in 21.2% of patients. Grade 3 noninfectious pneumonitis was observed only in one (3%) patient.

In another singe arm phase I study, addition of everolimus to vinorelbine and trastuzumab combination showed that ORR was 19.1% and median PFS was 30.7 weeks in patients with HER2-positive MBC who pretreated with trastuzumab [43]. Disease control rate was reported in 83.0% of patients. The most common adverse events were neutropenia (92%) and stomatitis (70%). Grade 3 or 4 neutropenia was reported in 14% of patients whereas grade 3 or 4 stomatitis was reported in 12% of patients in this single arm study.

A phase I/II study of trastuzumab in combination with everolimus, who progressed during trastuzumab treatment in patients HER2-positive MBC, showed a clinical benefit rate (CBR) in 34% (15% partial response, 19% stable disease) of patients with a median 4.1 months PFS [44]. The most common reported hematological toxicity was lymhopenia (26%) and the most common reported nonhematological toxicity was mucositis (34%). According to the biomarker analysis of

this study, patients with PTEN loss significantly demonstrated decrease OS, but no effect of PTEN loss on PFS was found. According to the analysis of PI3K pathway mutations, OS and PFS was not statistically affected with the PI3K mutational status.

In phase I study of weekly everolimus in combination with docetaxel in patients with heavily pretreated MBC showed that grade 3 or 4 neutropenia (73%) was seen in the majority of the patients [45]. Fatigue (40%) is the most common nonhematological toxicity. No partial or complete response was found but stable disease was seen in 53% of patients. Due to the increase risk of neutropenia and lack of efficacy of weekly everolimus with combination docetaxel, this study was terminated.

In phase I trial of everolimus combination with carboplatin in patients with pretreated MBC showed 21% partial response and 43% stable disease [46]. Most commonly seen grade 3 or 4 adverse events were leukopenia, thrombocytopenia and infection.

A phase Ib study of everolimus in combination with EGFR inhibitor erlotinib in heavily pretreated MBC patients showed partial response only in one (8.3%) patient and the remaining 11 patients (91.7%) had progressed in the first clinical evaluation [47]. Two patients had discontinued to trial due to the grade 3 stomatitis before the first clinical evaluation. The most common toxicities with the combination of erlotinib and everolimus were rash (16%), transaminase elevation (15%) and stomatitis (13%). Generally the combination of erlotinib and everolimus was well-tolerated, but the clinical activity was reported poorly in this heavily pretreated MBC patients.

In HER2-negative MBC patients, a phase I trial of cisplatin, paclitaxel and everolimus combination showed that median PFS was 5 months [48]. In this study 70% of patients were triple-negative and all of them had visceral disease. Complete response, partial reponse and stable

disease was observed in 7.7%, 15.4% and 53.8%, respectively. The most common adverse events were alopecia (100%) and neutropenia (28%).

The resulted phase I trials were summarized in Table 1.

Phase II trials of everolimus in breast cancer

In a phase II randomized study of comparing everolimus 10 mg daily versus 70 mg per week in patients who received none or one prior chemotherapy in MBC showed that treatment discontinuation was higher in daily schedule compared to weekly treatment (27% vs %13) [49]. In this study complete response was seen in 12% of patients, partial response was seen in 28.2% of patients and 30.6% of patients had stable disease. The median duration of complete response was 13.1 months whereas 3.7 months in partial responser patients. The most common adverse events were fatigue (67.3%), neutropenia (61.2%) pneumonitis (34.7%) and infection (26.6%). Drug related serious adverse events more frequently observed in daily schedule compared to weekly schedule.

In a randomized phase II TAMRAD (Tamoxifen Plus Everolimus) study, efficacy and safety of everolimus in combination with tamoxifen in patients hormone receptor-positive, HER2-negative MBC who exposure to prior aromatase inhibitors was investigated. In TAMRAD study, CBR was 61% in combination arm whereas it was 42% in tamoxifen monotherapy arm (P = 0.04) [50]. In the subgroup analysis according to the secondary hormone resistance, combination arm had a higher CBR compared to tamoxifen arm alone (74% vs 48%). Seventy-eight percent of patients had bone metastases and 53% of patients had visceral metastases. In this study, time to progression (TTP) was 4.5 months and 8.6 months in tamoxifen arm and combination arms, respectively (P = 0.002). Median OS was not reached with the combination arm, whereas OS was 32.9 months tamoxifen arm (55% RR of death, p = 0.007). Most commonly observed

nonhematological adverse events with combination arm were fatigue (72%), stomatitis (56%), rash (44%) and anorexia (43%). Most commonly reported hematological adverse events with combination arm were decreased hemoglobin (69%) and leukopenia (54%). Grade 3 or 4 adverse events were similar between two arms (P = 0.20). The overall incidence of serious adverse events were similar in both groups; 32% in each group. This study showed that tamoxifen plus everolimus increased CBR, TTP and OS compared to tamoxifen monotherapy in aromatase inhibitor resistant postmenopausal MBC patients.

In a randomized phase II study, the efficacy and safety of neoadjuvant letrozole plus everolimus or placebo study in patients with operable ER-positive breast cancer was investigated. In this neodajuvant study, the response rate was significantly higher in the combination with everolimus arm compared to letrozole monotherapy (68.1% vs 59.1%; one sided P = 0.06) [51]. The response rate with ultrasound evaluation also significantly higher in the everolimus arm compared to placebo arm (58% vs 47%, P = 0.03). Complete response was observed in 13.4% of patients in letrozole plus everolimus arm, whereas in 9.1% of patients in letrozole plus placebo arm. Also the mean reduction of Kİ67-positive tumor cells were statisticaly higher in the everolimus arm compared to placebo arm (90.7% vs 74.8%; P = 0.0002). In the safety analysis; dose reduction or interruption was reported in 52.9% and 7.6% of patients treated with everolimus and placebo arms, respectively. Stomatitis (36.5%) and rash (20.4%) were the most commonly reported adverse events in the everolimus arm. Grade 3 or 4 adverse events were reported in 22.6% and 3.8% who received everolimus and placebo, respectively. As a result of this study everolimus increased the efficacy of letrozole in the neoadjuvant treatment of ER-positive breast cancer.

A phase II study of weekly cisplatin/paclitaxel plus everolimus in HER2-negative MBC patients showed partial response in 20% of patients and stable disease in 38.2% of patients [52]. In this study, 63% of patients were triple-negative and 81% of patients had visceral disease. Sixty-two percent of patients had prior at least three prior regimen chemotherapy regimens in the metastatic setting. Median TTP was 6 months in the evaluable patients. The most commonly reported adverse events were anemia (72%), trombocytopenia (56%) and neutropenia (44%). Significant antitumor activity was seen with everolimus in this heavily pretreated MBC patients.

In a phase II trial of everolimus and carboplatin in patients with triple-negative MBC showed that median CBR was 13 weeks (6-74 weeks) with the combination treatment [53]. Median TTP was 85 days with combination arm. The patients were pretreated with prior 0-3 settings of chemotherapy for metastatic disease. The most common grade 3 or 4 adverse events were trombocytopenia (22.7%) and neutropenia (18.2%). This study demonstrated that everolimus had clinical benefit in triple-negative patients.

In an open label phase II randomized study, standard neoadjuvant chemotherapy with paclitaxel followed by 5-fluorouracil-epirubicin-cyclophosphamide (T-FEC) was compared with paclitaxel and everolimus combination followed by FEC (TR-FEC) regimen in women with triple-negative breast cancer [54]. Clinical endpoints were response rate, pathological complete response (pCR) rate and toxicity. Response rates with ultrasound were 47.8% and 29.6% in TR-FEC and T-FEC regimens, respectively (one sided P = 0.15). Most commonly reported grade 3 or 4 adverse events were leukopenia (17%), anemia (13%) and vomiting (13%) in the everolimus arm. The toxicity profile did not significantly differ in two treatment arms. As a result, the response rate was higher in everolimus arm, but not significantly in triple-negative breast cancer patients in the neoadjuvant setting.

In another phase II study of everolimus in combination with letrozole in postmenopausal ER-positive MBC after failure of hormonal treatment showed partial response in 22% and stable disease in 28% of patients in the preliminary results of 24 patients [55]. Median TTP was 4 months. The most common reported adverse events were fatigue (90%) and rash (70%). This single arm study showed that letrozole combination with everolimus had significant activity in ER-positive postmenopausal MBC patients.

The resulted phase II trials were summarized in Table 2.

Phase III trials of everolimus in breast cancer

In a phase III randomized BOLERO-2 (The Breast Cancer Trials of Oral Everolimus-2) trial, the efficacy of everolimus plus exemestane was compared with exemestane plus everolimus in postmenopausal hormone receptor-positive advanced breast cancer who had recurrence or progression while receiving nonsteroidal aromatase inhibitor [23]. In this study, 56% of patients had visceral disease whereas 76% had bone metastases. All patients were HER2-negative. The primary endpoint of BOLERO-2 trial was PFS. Median PFS was 6.9 months in exemestane plus everolimus and 2.8 months in exemestane plus placebo arm (P<0.001). According to the central assesment, median PFS was 10.6 months and 4.1 months in everolimus and placebo arms, respectively (P<0.001). Response rates were significantly higher in the combination arm (9.5% vs 0.4%; P<0.001). The most common grade 3 or 4 adverse events in everolimus group were stomatitis (8%) anemia (6%) and dyspnea (4%). In the presented final PFS analysis of BOLERO-2 trial at a median 18 month follow-up, everolimus plus exemestane compared to exemestane plus placebo had significantly higher PFS (7.8 months vs 3.2 months; P<0.0001) [56]. In the presented final analysis; central assesment median PFS was 11.0 months and 4.1 months in everolimus and placebo arms, respectively (P<0.001). Also fewer deaths were reported with

everolimus plus exemestane arm compared to exemestane plus placebo arm (25.4% vs 32.2%). In the safety analyses of everolimus in the elderly (over age 65); elderly patients in the everolimus arm had similar or marginally lower incidence of stomatitis (52.1%), rash (32.3%) and pneumonitis (14.6%) compared with the overall population [57]. In the subgroup analyses of BOLERO-2, treatment with everolimus significantly improved median PFS in Asian patients [58]. Median PFS was 8.4 months in everolimus plus exemestane arm, 4.1 months in the exemestane plus placebo arm. The most common grade 3 and 4 events were similar in both Asian and non-Asian groups. But pneumonitis was higher in the everolimus plus exemestane arm in Asian patients, compared to non-Asian patients (23.5% vs 14.1%). In BOLERO-2 trial, to measure health-related quality of life European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) was assessed at baseline and in every 6 weeks until progression [59]. Time to deterioration (TTD) in health-related quality of life was 8.3 months in everolimus and exemestane arm whereas it was 5.8 months in the exemestane plus placebo arm (P = =0.008). Because of preclinical studies had shown that mTOR inhibition was associated with decreased osteoclast activity and survival; effect of everolimus on bone marker levels was investigated in BOLERO-2 trial [60]. Bone turnover marker levels were measured at baseline and 6 and 12 weeks after treatment initiation. As a result cumulative incidence of bone disease progression was compared between exemestane plus everolimus and exemestane alone arms. Bone turnover marker levels significantly increased in exemestane alone arm whereas bone turnover marker levels decreased with combination of exemestane and everolimus. Bone lesions specific progression (new bone lesions or progression of bone metastases) was significantly decreased with everolimus and exemestane arm by week 12. Furthermore, two times more benefit was observed in bone disease specific progression by 30th

week and similarly progressive disease in bone decreased beyond week 30 with everolimus combination.

In another phase III study (GeparQuinto study 44; GBG 44) of patients with HER2-negative tumors were randomized to 4 cycles of neoadjuvant epirubicin-cyclophosphamide with or without bevacizumab. Nonresponders of the 4 cycles of chemotherapy were then randomized to paclitaxel with or without everolimus [61]. The aim of GBG-44 study was to compare the pCR rates of neoadjuvant chemotherapy with or without everolimus. In 3.6% of patients in paclitaxel plus everolimus pCR was observed, whereas pCR was 5.6% in paclitaxel alone arm. The pCR rates in both groups were not significantly differ (P = 0.34). Adverse events were more frequently observed in everolimus arm. The most commonly observed adverse events in everolimus plus paclitaxel arm were leukopenia (91.3%) and anemia (89.6%). Neutropenia, leukopenia, trombocytopenia, diarrhea, allergic reactions, rash, fever, infection and elevated AST were observed significantly higher in the everolimus plus paclitaxel arm compared to paclitaxel alone. In this study addition of everolimus to paclitaxel did not improve the pCR in HER2-negative breast cancer.

Recently reported randomized phase III study (BOLERO 3) was designed to compare the efficacy of everolimus plus trastuzumab and vinorelbine with trastuzumab and vinorelbine combination in trastuzumab-resistant HER2-positive MBC patients [62]. Patients were randomized to placebo or everolimus 5 mg/d with weekly trastuzumab and vinorelbine. The primary endpoint was PFS. In this study 100% of patients were previously treated with taxanes and trastuzumab and 28% of patients were previously treated with lapatinib. Median PFS was 7.0 months in the everolimus arm whereas 5.8 months in the placebo arm (P<0.01). The ORR rate in the everolimus arm was 40.8% compared to 37.2% in the placebo arm, which did not reach

significance (P=0.21). Clinical benefit rates were 59.2% and 53.3% for the everolimus and placebo arms, respectively (P=0.09). The adverse event profile on everolimus arm was unsurprising despite 5 mg daily dose. In everolimus arm, a higher rate of stomatitis, pyrexia, and decreased appetite was reported in everolimus arm compared to placebo arm. In addition, hematologic toxicities like neutropenia, anemia, febrile neutropenia, and thrombocytopenia were elevated in the everolimus arm, as well. Despite the increased rate of adverse events, the time to deterioration of global health status was not significantly different in the two arms. But the additional toxicity of everolimus arm did not significantly impact quality of life. The important question of BOLERO-3 trial is whether the combination of vinorelbine and trastuzumab is an acceptable combination clinically. Other important questions of BOLERO-3 trial include the "what the optimal dose of everolimus is and which patients can benefit with the addition of everolimus in the subgroup analyses".

Ongoing Clinical trials with everolimus in breast cancer

BOLERO-1 trial is a randomized phase III, double blind, placebo controlled study of everolimus in combination with trastuzumab and paclitaxel as first line therapy in patients with HER2-positive locally advanced or metastatic breast cancer [63]. In BOLERO-1, 719 patients have been randomly assigned by 2:1 (everolimus vs control). The primary endpoint of BOLERO-1 study is PFS and secondary endpoints are OS, ORR and CBR. The estimated study completion date is December 2013.

BOLERO-4 is a single arm, open-label, phase II study of everolimus plus letrozole as first-line treatment of patients with ER-positive MBC [64]. Patients will receive everolimus (10 mg/d) and letrozole (2.5 mg/d) until first disease progression. In BOLERO-4, 200 postmenopausal ER-positive, HER2-negative MBC patients' enrollment were estimated. The primary endpoint is PFS and secondary endpoints are

second-line PFS, OS, Objective response rate, CBR and safety. Estimated study completion date is February 2016.

BOLERO-6 is a randomized, 3-arm, phase 2 study of everolimus plus exemestane versus everolimus or capecitabine monotherapy in hormone receptor-positive, HER2-negative advanced breast cancer [65]. In this study, estimated 300 patients will be randomized to receive either everolimus (10 mg/d) alone or exemestane (25 mg/d) plus everolimus (10 mg/d) combination therapy or capecitabine (1,250 mg/m^2 twice daily for 14 d/3-wk cycle) alone until disease progression. The primary endpoint is PFS and secondary endpoints are OS; objective RR, CBR, safety and quality of life. Estimated study completion date is April 2015.

VICTORIA trial is phase II study of vinorelbine in combination with everolimus or vinorelbin monotherapy alone for second-line treatment of advanced breast cancer [66]. The primary endpoint is PFS and secondary endpoints are safety, rate of PFS at 6 months, OS and response rate (RR). In VICTORİA trial, 166 postmenopausal HER2-negative MBC patients' enrollment were estimated. Estimated study completion date is June 2015.

BRE-43 trial is single arm, phase II study of combined fulvestrant and everolimus in advanced and metastatic breast cancer who relapsed or metastatic disease progression within aromatase inhibitor use [67]. Primary endpoint of this study is TTP and secondary endpoints are safety, RR, CBR and biomarker analysis. Estimated enrollment of this study is 44 postmenopausal hormone receptor-positive advanced and metastatic breast cancer patients. Estimated study completion is June 2013.

GeparQuinto is a phase III trial that exploring the integration of bevacizumab, everolimus and lapatinib into current neoadjuvant chemotherapy regimes for primary breast cancer [68]. In this parallel group study 6 different arms were determined; arm 1; epirubicin-

cyclophosphamide(EC)/docetaxel (D), arm 2; EC/D+bevacizumab, arm 3; paclitaxel alone, arm 4; paclitaxel+everolimus, arm 5; EC/D+trastuzumab and arm 6; EC/D+lapatinib. Primary endpoint is pCR and secondary endpoints are compliance, breast conservation rate, OS and locoregional and distant disease free survival. Estimated enrollment of this study is 2600 locally advanced or high risk breast cancer patients and the estimated study completion date is December 2015.

SWOG/NSABPS1207 study is a phase III randomized, placebo-controlled clinical trial evaluating the use of adjuvant endocrine therapy of everolimus in patients with high-risk, hormone receptor-positive HER2-negative breast cancer [69]. The aim of this study is to prove the efficacy of everolimus in the adjuvant setting. All patients must have completed surgery, adjuvant/neoadjuvant chemotherapy and radiation therapy before registration. The study plans to randomize 3,500 patients over a 3.5-year. The primary endpoint of this study is disease free survival (DFS) after two years of treatment thus the expected trial duration from activation to reporting of DFS is about 7 years. Secondary endpoints are OS, event free survival, incidence of secondary cancers, impact of subgroups and safety. The estimated study completion date is January 2018.

The ongoing selected phase II-III trials were summarized in Table 4.

Temsirolimus

Temsirolimus is an inhibitor of mTOR and it inhibits the synthesis of cell cycles that regulate proliferation, growth, and survival of tumor cells . Treatment with temsirolimus leads to cell cycle arrest in the G1 phase, and also inhibits tumor angiogenesis by reducing synthesis of VEGF [70]. Phase II study of temsirolimus in heavily pretreated locally advanced or MBC patients aimed to show efficacy [71]. A total of 109 patients were treated either 75 mg or 250 mg

temsirolimus per week. The median TTP was 12 weeks with 9.2% ORR. The most common adverse events with temsirolimus were mucositis (70%), maculopapular rash (51%) and nausea (43%). A phase II randomized, 3 arm study in 92 women compared the efficacy and safety of letrozole alone or combination with 10 mg or 30 mg daily temsirolimus [72]. Patients in the temsirolimus group had significantly longer PFS compared to letrozole arm alone (18.0 versus 9.5 months, respectively).

Recently randomized phase III placebo-controlled trial (HORIZON study) of letrozole plus oral temsirolimus for first line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer was reported. In HORIZON study, median PFS was 9.0 months and 8.9 months in letrozole plus temsirolimus and letrozole plus placebo arms, respectively (P = 0.25) [73]. According to the subgroup analyses of age ≤ 65 years; median PFS was 9.0 months and 5.6 months in letrozole plus temsirolimus and letrozole plus placebo arms, respectively (P =0.009). The most commonly reported adverse events in temsirolimus arm were asthenia (27%), diarrhea (21%) and headache (19%). In conclusion of this study, adding temsirolimus to letrozole did not improve median PFS in the first line hormonal treatment of postmenopausal ER-positive MBC. In this trial, temsirolimus failed to show efficacy in aromatase inhibitors-naive postmenoposal breast cancer patients whereas adding everolimus to exemestane had significant PFS benefit in postmenopausal hormone receptor-positive advanced breast cancer who had recurrence or progression while receiving nonsteroidal aromatase inhibitor. The lack of benefit with temsirolimus in this trial confirms that acquired resistance to aromatase inhibitors can determine response the aromatase inhibitors.

Sirolimus

Sirolimus, also known as rapamycin, inhibits the response to interleukin-2 and thereby blocks activation of T and B cells. The mode of action of sirolimus is to bind the cytosolic protein FK-binding protein 12 (FKBP12) thus the sirolimus-FKBP12 complex inhibits the mTOR pathway by directly binding the mTORC1 [74]. Randomized phase II study of hormone receptor-positive, HER2-negative MBC patients was designed to show efficacy of addition of sirolimus to tamoxifen versus tamoxifen [75]. In this study, 400 patients were included. Response rate was observed in 38.8% and 4.1% in sirolimus plus tamoxifen and tamoxifen arms, respectively (P=0.00018). In tamoxifen plus sirolimus arm median TTP was 11.7 months whereas it was 3.3 months,in tamoxifen alone arm (P=0.0023). The patients who had progression of disease on tamoxifen within 6 months or prior exposure to aromatase inhibitors had nonsignificantly higher TTP with addition of sirolimus to tamoxifen (TTP; 7.4 vs 2.2 months). In patients who had no prior exposure to aromatase inhibitors, TTP was 16 months and 9 months in sirolimus plus tamoxifen and tamoxifen alone arms (P=0.0028). The only phase II study of sirolimus concluded that sirolimus and tamoxifen combination was effective.

Ridaforolimus and deferolimus are also rapamycin analogs but the clinical datas from these new mTOR inhibitors in breast cancer are insufficient until now [74].

Discussion

It is well known that breast cancer cells can develop resistance to standard hormonal treatment and chemotherapy with the activation of mTOR pathway which supported by resulted preclinical and clinical studies. In vitro studies showed that mTOR activation was related with HER2 overexpression and responsible for the HER2-positive breast cancer progression. mTOR activation is also responsible mechanism of chemotherapy resistance in triple-negative breast cancers. Also, preclinical studies have shown that mTOR activation and Akt signaling

upregulation was one of the responsible mechanism of endocrine treatment resistance and mTOR inhibitors may contribute to break resistance of hormonal treatment. As a result, both in vivo and in vitro studies showed that antitumor activity of everolimus was reported in all subgroups of breast cancer.

There is an unmet therapeutic need in all subgroups of MBC. Preclinical studies showed that mTOR activation can be the responsible mechanism in all subgroups of the breast cancer. Results of both TAMRAD and BOLERO-2 studies have showed that, mTOR inhibition in combination with endocrine therapy can be a new treatment strategy for hormone-receptor positive MBC patients who resistant to aromatase inhibitors. In BOLERO-2 study, time to deterioration in health-related quality of life was also significantly higher in everolimus and exemestane arm compared to exemestane plus placebo arm. Recently completed BOLERO-3 study showed that, mTOR inhibition in combination with trastuzumab and vinorelbine treatment significantly improved PFS compared to trastuzumab and vinorelbine combination in trastuzumab-resistant MBC patients.

Pharmacodynamic studies suggested that 10 mg daily dose is the standard dose of everolimus in cancer patients [18]. In a randomized phase II study comparing two schedules of everolimus; response rate was reported in 12% of 10 mg daily whereas no response was observed in everolimus 70 mg per week arm [49]. But there is no randomized study that compare the efficacy between 5 mg or 10 mg daily everolimus. Generally adverse effects of everolimus was well tolerated as predicted from preclinical and early clinical studies. Clinically adverse events of everolimus may be the class effects of mTOR inhibitors. The most common reported adverse events with everolimus were stomatitis, rash, fatigue, diarrhea, asthenia, cough, pyrexia and hyperglycemia [23]. The most common grade III or IV adverse events were stomatitis, anemia,

hyperglycemia, fatigue and pneumonitis. In a phase I study of everolimus grade III or IV neutropenia, lymphopenia, leukopenia, stomatitis and metabolic disorders were seen more commonly in everolimus 10 mg daily arm compared to 5 mg arm [42]. In a recent reported phase III BOLERO-3 study, the adverse event profile of everolimus arm was unsurprising despite 5 mg daily dose [62]. The adverse event profile of 5 mg daily everolimus was similar to previous everolimus studies. Despite the pharmacodynamic studies suggested that 10 mg daily dose is the standard dose of everolimus, still there is not enough data about what the optimal dose should be in breast cancer patients.

Stomatitis is one of the most common adverse event of everolimus as other mTOR inhibitors. Grade III or IV stomatitis was reported in 8% of patients in everolimus arm of BOLERO-2 trial [23]. The mTOR-induced stomatitis can be treated with topical steroids and mouthwashes as like chemotherapy-induced stomatitis [76]. Everolimus induced-stomatitis is usually dose related and dose modifications may be necessary for grade II or III stomatitis [42,77]. Everolimus should be discontinued in the event of grade IV stomatitis. Noninfectios pneumonitis, also one of the most common adverse event, is a class effect adverse event of mTOR inhibitors. Grade III or IV noninfectious pneumonitis reported in 4% of patients in everolimus arm of BOLERO-2 trial [23]. Patients may be asymptomatic or have nonspecific respiratory symptoms. Fever sometimes accompanied to respiratory symptoms making distinction difficult from infection causes. Symptomatic cases may be mild or moderate. In grade II or III pneumonitis everolimus should be stopped temporarily until symptoms relieves. Corticosteroids may be necessary for patients with moderate and severe symptoms, and everolimus should be permanently discontinued in grade IV pneumonitis patients [78]. Steroids with 1 mg/kg daily dose can be used in symptomatic grade II-IV patients after infection ruled out until the symptoms resolved [79]. As a class effect,

everolimus has immunosuppressive properties, thus evrolimus may increase the incidence of opportunistic infections and may reactivate the previous infections [77]. The risk of bacterial and fungal infections also increased with everolimus. A complete medical history of the patients should be taken before beginning of everolimus because hepatit B activatiaon can be seen in up to 20 percent of patients with a 5-40% mortality rate [80,81].

All mTOR inhibitors did not show similar activity in hormone receptor-positive MBC. Despite the promising results of everolimus, temsirolimus has shown only modest activity in the treatment of hormone receptor-positive breast cancer patients. Results of HORIZON trial showed no improvement with the addition of temsirolimus to letrozole in postmenopausal MBC [73]. The exact disparity between the results of temsirolimus and everolimus is not clear, but it may be due to different selection criterias of this two trials. In HORIZON trial, none of the patients had received aromatase inhibitors as adjuvant therapy, whereas patients in BOLERO-2 trial had required progression during or completing adjuvant aromatase inhibitor or within 1 months if the patient in the metastatic setting [23]. As a result, in HORİZON trial aromatase inhibitor-naive patients were included into the study, whereas patients who treated with prior non-steroidal aromatase inhibitors were included to the BOLERO-2 trial. The difference of eligibility criteria of both trials can explain the difference of response rates in everolimus and temsirolimus trials. Prior exposure to aromatase inhibitors associated with intrinsic tumor factors and can increase the response of mTOR inhibitors [82]. Temsirolimus is a highly specific mTORC1 inhibitor that does not fully suppress the phosphatidyl inositol kinase pathway (PI3K) can explain why temsirolimus failed to show response in patients postmenopausal breast cancer.

Currently data in phase III trials with everolimus is promising, especially in hormone-receptor-positive MBC. It is important to know that which patients will most likely benefit from

these therapies is important. Thus, we need the subgroup biomarker analysis to plan targeted therapies more correctly.

Conclusion

Recent trials showed that everolimus, has produced promising anti-tumor activity in combination with trastuzumab in HER2-positive metastatic breast cancer and in combination with exemestane in hormone receptor-positive metastatic breast cancer who had recurrence or progression while receiving nonsteroidal aromatase inhibitor. With the results of ongoing studies with everolimus in the future may tending towards of these agents in earlier stages of the disease, namely in the adjuvant and neoadjuvant settings.

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Table 1. Phase I clinical trials of Everolimus in breast cancer**.**

Trial Name	Number of patients	Study design	Cancer Type	Primary Endpoint	Comment
Awada et al.[41]	33	MBC; Single arm; L+E	MBC	Safety	Stomatitis: 50% RR >6 months; 38.9%
Andre et al.[42]	18	HER2-positive MBC pretreated with trastuzumab Single arm; P+T+E	MBC	DLT, safety, RDI and ORR	Neutropenia ; 52% ORR; 44.0% PFS; 34 weeks
Jerusalem et al. [43]	50	HER2-positive MBC pretreated with trastuzumab Single arm; V+T+E	MBC	DLT and safety	Neutropenia; 92% Stomatitis; %70 ORR; 19.1% PFS; 30.7 weeks
Morrow et al. [44]	47	HER2-positive MBC pretreated with trastuzumab Single arm; T+E	MBC	Safety and efficacy (CBR, PFS)	Mucositis (34%)[44] CBR; 34% PFS: 4.1 months
Moulder et al.[45]	15	Refractory to standart CT of MBC Single arm; D+E	MBC	Safety	Neutropenia ; 73% Fatigue; 40% SD; 53%
Schwarzlose-Schwarck et al.[46]	15	Pretreated MBC Single arm; C+E	MBC	Safety	PR; 21% SD; 43%
Mayer et al [47].	14	A Pretreated MBC Single arm; Erlotinib+E	MBC	Safety	Rash (16) PR; 8.3%
Mayer et al [48].	16	HER2 negative MBC Single arm; Cisplatin+P+E	MBC	Safety	Alopecia; (100%) Neutropenia (28%) PFS; 5 months

Abbreviations: MBC; metastatic breast cancer, DLT; dose limiting toxicity, RDI; relative dose intensity, T; trastuzumab, D; docetaxel, C; carboplatine, E; everolimus, L; letrozole, P; paclitaxel, T; trastuzumab, V; vinorelbine, CR; complete response, RR; response rate, SD; stable disease, PR; partial response, ORR; overall response rate, PFS; progression free survival, CBR; clinical benefit rate

Table 2. Phase II clinical trials of Everolimus in breast cancer**.**

Trial Name	Number of patients	Study design	Cancer Type	Primary Endpoint	Comment
Ellard et al (NCIC CTG IND.163)[49]	49	No or one prior chemotherapy for MBC, Single arm; E	MBC	Efficacy; ORR	CR; 12% PR; 28.2%
Bachelot et al (TAMRAD)[50]	111	Hormone receptor (+), HER2 (-) MBC (Prior exposure to AIs) Arm 1; E+T Arm 2; T+Pla	MBC	Efficacy; RR	CBR; E+T.; 61% T.; 42% (P= 0.04) TTP; E+T; 8.6 months T; 4.5 months
Baselga et al (NCT00107016)[51]	270	ER-positive operable breast cancer, neoadjuvan Arm 1; L+Pla. Arm 2; L+E	EBC	DLT and safety	RR; L+E; 68.1% L+Pla; 59.1 (One sided P =0.06)
Mayer et al[52]	55	HER2-negative MBC heavily pretreated Single arm; P+C+E	MBC	Safety	Anemia (72%) PR; 20% SD; 38.2% TTP; 6 months
Singh et al[53]	23	Triple-negative MBC Single arm; Car+E	MBC	CBR and safety	CBR; 13 weeks Trombocytopenia; 22.7%
Gonzalez-Angulo	62	Triple-negative operable breast	EBC	RR, pCR and	RR; TR-FEC; 47.8%

et al[54]		cancer, neoadjuvant Arm 1; TR-FEC Arm 2; T-FEC		toxicity	T-FEC; 29.6% (one sided P = 0.15)
Safra et al.[55]	24	ER-positive MBC after failure of hormonal treatment Single arm; L+E	MBC	ORR, PFS, OS and safety	ORR; 50% TTP; 4 months Fatigue; 90%

Abbreviations: EBC; early breast cancer, MBC; metastatic breast cancer, D; docetaxel, C; cisplatin, Car; carboplatin, E; everolimus, L; letrozole, Pla; placebo P; paclitaxel, T; tamoxifen, T-FEC; paclitaxel followed with 5-fluorouracil-epirubicin-cyclophosphamide, TR-FEC; paclitaxel plus everolimus followed with 5-fluorouracil-epirubicin-cyclophosphamide, CR; complete response, RR; response rate, SD; stable disease, PR; partial response, ORR; overall response rate, PFS; progression free survival, CBR; clinical benefit rate, OS; overall survival, TTP; time to progression

Table 3. Phase III clinical trials of Everolimus in breast cancer**.**

Trial Name	**Number of patients**	**Study design**	**Cancer Type**	**Primary Endpoint**	**Comment**
Baselga et al (BOLERO-2)[23]	724	Hormone-receptor-positive MBC Arm 1; Exe+E Arm 2; Exe+Pla	MBC	PFS	PFS; Exe+E; 10.6 m, Exe+Pla; 4.1 m
Noguchi et al (Subgroup of BOLERO-2)[58]	143	Hormone-receptor-positive MBC İN Asian patients Arm 1; Exe+E Arm 2; Exe+Pla	MBC	PFS	PFS; Exe+E; 8.4 m, Exe+Pla; 4.1 m
Burris et al (subgroup of BOLERO-2)[59]	724	Hormone-receptor-positive MBC Arm 1; Exe+E Arm 2; Exe+Pla	MBC	PFS HRQOL (secondary end point)	TTD; Exe+E; 8.3 m, Exe+Pla; 5.8 m (P = 0.008)
Huober et al (GBG-44)[61]	397	HER2-negative breast cancer after 4 cycles of EC±B;	EBC	pCR	pCR; P+E; 3.6% P; 5.6% (P = 0.34)

		Arm 1; P+E Arm 2; P+Pla			
O'Regan et al (BOLERO-3)[62]	569	HER-2 positive MBC resistant to trastuzumab; Arm 1; T+V+E Arm 2; T+V+Pla	MBC	PFS	PFS; T+V+E; 7.0 m T+V+Pla; 5.8 m

Abbreviations: BOLERO; The Breast Cancer Trials of Oral Everolimus-2, GBG 44; GeparQuinto study 44; EBC; early breast cancer, MBC; metastatic breast cancer, HRQOL; health-related quality of life, TTD; time to deterioration, T; trastuzumab, E; everolimus, EC; epirubicin/cyclophosphamide, Exe; Exemestane, P; paclitaxel, T; trastuzumab, V; vinorelbine, Pla; placebo pCR; pathological complete response, PFS; progression free survival

Table 4. Ongoing Phase II-III clinical trials of Everolimus in breast cancer**.**

Trial Name	**Phase**	**Study design**	**Cancer Type**	**Primary Endpoint**	**Comment**
BOLERO-1[63] (Est. n=719)	III	1st line treatment of HER2-positive Advanced or MBC Arm 1; P+T+E Arm 2; P+T+Pla	MBC	PFS	NCT00876395 ESCD: 12/2013
BOLERO-4[64] (Est. n = 200)	II	1ST line of ER-positive MBC Single arm; L+E	MBC	PFS	NCT01698918 ESCD: 02/2016
BOLERO-6[65] (Est. n = 300)	II	1st line of hormone receptor- positive MBC; 3 arms Arm 1; E alone, Arm 2; E+Exe. Arm 3; C alone	MBC	PFS	NCT01783444 ESCD: 04/2015
VICTORİA[66] (Est. n = 166)	II	2nd line of HER2-negative MBC; Arm 1: E+ V	MBC	PFS	NCT01520103 ESCD: 06/2015

		Arm 2: V alone			
BRE-43[67] (Est. n = 44)	II	2nd line of ER-positive MBC; Single Arm; F+E	MBC	TTP	NCT00570921 ESCD: 06/2013
GeparQuinto[68] (Est. n = 2600)	III	Locally advanced BC Neoadjuvant 6 arms; Arm 1; EC/D, Arm 2; EC/D+B, Arm 3; P alone Arm 4; P+E Arm 5; EC/D+T Arm 6 EC/D+L	LABC	pCR	NCT00567554 ESCD: 12/2015
SWOG/NSABP S1207[69] (Est. n = 3500)	III	High risk, hormone receptor-positive HER2-negative BC Arm 1; Hormonal treatment+E Arm 2; Hormonal treatment+Pla.	EBC	DFS	NCT01805271 ESCD: 01/2018

Abbreviations: BC; breast cancer, EBC; early breast cancer, LABC; locally advance breast cancer, MBC; metastatic breast cancer, ESCD; estimation study completion date, Est n; estimated enrollment of patients, B; bevacizumab, C; capacitabine, D; docetaxel, E; everolimus, EC; epirubicin/cyclophosphamide, Exe; exemestane, F; fulvestrant, L; lapatinib, P; paclitaxel, Pla; placebo, T; trastuzumab, V; vinorelbine, DFS; disease free survival, PFS; progression free survival, pCR; pathological complete response, TTP; time to progression