Breast Cancer: Basic and Clinical Research

REVIEW

Combining mTOR Inhibitors with Chemotherapy and Other Targeted Therapies in Advanced Breast Cancer: Rationale, Clinical Experience, and Future Directions

Denise A. Yardley

Sarah Cannon Research Institute, Nashville, Tennessee, USA. Corresponding author email: dyardley@tnonc.com

Abstract: Improvements in survival of patients with breast cancer have been attributed to the development of agents that target key components of dysregulated pathways involved in oncogenesis and progression of breast cancer. Aberrant mammalian target of rapamycin (mTOR) activation has been implicated in oncogenesis, angiogenesis, and the development of estrogen independence and resistance to chemotherapy in breast tumors. Several mTOR inhibitors (sirolimus, everolimus, temsirolimus, and ridaforolimus) have demonstrated antitumor activity in breast cancer cells. Combining mTOR inhibitors with endocrine therapies has demonstrated clinical antitumor activity in patients with metastatic breast cancer. In addition, mTOR inhibitor combinations with various targeted biologic agents or cytotoxic chemotherapeutic agents are being examined in more than 40 clinical trials with some early promising results. Combination therapies targeting multiple components of these central signaling pathways may be an optimal treatment strategy for patients with advanced breast cancer.

Keywords: mTOR, hormone receptor, HER2, advanced breast cancer

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Introduction

Improvements in overall or disease-free survival of patients with breast cancer have been attributed in part to the identification of dysregulated cell signaling pathways and the recent development of therapies that target key components of these pathways.[1] Some of these targets include vascular endothelial growth factor (VEGF) and its receptors (VEGFRs), epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2), and insulin growth factor receptors (IGFRs).[2] Monotherapy drug strategies aimed at these targets have yielded modest results in breast cancer trials, and many patients subsequently develop resistance to these agents. Despite these limitations, the role of cell signaling among these various pathways is clearly evident. Therapeutic agents in combination that target the aforementioned growth factors and/or their receptors represent an area of active investigation that has produced promising results, with some notable exceptions.[2]

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that integrates multiple signals from growth factors and hormones[3,4] and plays a central role in the control of cell growth, proliferation, and angiogenesis.[4,5] Aberrant mTOR activation has been implicated in oncogenesis, angiogenesis, and the development of drug resistance in tumor cells.[6–9] A high proportion of breast tumors exhibit constitutive activation of the mTOR pathway.[10] Convergence of multiple signaling pathways toward a central mediator, such as mTOR, lends itself to the hypothesis that targeting multiple pathways, including mTOR, with different classes of agents may be a more effective treatment strategy than monotherapy approaches.[11] Several mTOR inhibitors have been developed and evaluated in combination with chemotherapy, hormonal therapies, and other targeted biologic agents to investigate the utility of combination therapy for patients with advanced breast cancer.

Overview of the mTOR Pathway

Endocrine and growth factor receptors involved in the pathogenesis of breast cancer have been shown to activate mTOR through phosphoinositide 3-kinase (PI3K)/Akt signaling (Fig. 1).[12,13] Multiple upstream signaling components of this pathway, such as the estrogen receptor (ER),[14] the receptor tyrosine kinase HER2,[15] VEGFR, IGF-1R, phosphatase and tensin homolog (PTEN), Akt (also known as protein kinase B),[16] PI3K, and the PI3K catalytic subunit (PI3KCA),[17] become deregulated in breast cancer and are thought to play a role in its development and progression.[18,19] Therefore, inhibiting mTOR potentially interferes with breast cancer progression at multiple levels. Preclinical evidence demonstrates that the PI3K/Akt/mTOR pathway is involved in the response of breast cancer cells to hormonal therapies,[13] chemotherapy,[20] and targeted agents.[21,22] Members of the EGFR family, including HER2, use the PI3K/Akt/mTOR pathway to promote cell growth and survival.[22] Overexpressed in approximately 20% of invasive breast cancers and associated with a poor prognosis,[23,24] HER2-mediated activation of the PI3K/Akt/mTOR pathway is predictive of tumor progression in breast cancer[22] and has been implicated in angiogenesis and metastasis of breast cancer cells.[15] Trastuzumab, a monoclonal antibody that binds to the extracellular domain of HER2, has been shown to diminish HER2-mediated signaling,

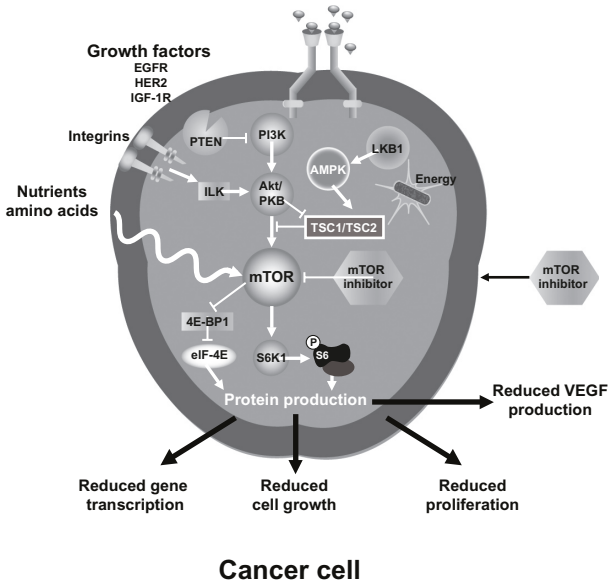

Figure 1. The PI3K/Akt/mTOR pathway.[13]
From Lane H, Lebwohl D. Future directions in the treatment of hormone-sensitive advanced breast cancer: the RAD001 (everolimus) letrozole clinical program.
Reprinted with permission from Elsevier.[13]
Abbreviations: 4E-BPs, 4 eukaryotic binding proteins; EIF4K, eukaryotic initiation factor 4K; mTORC, mammalian target of rapamycin complex; PTEN, phosphatase and tensin homolog; S6K1, ribosomal protein S6 kinase 1; S6, ribosomal protein S6; TSC1, tuberous sclerosis complex1; TSC2, tuberous sclerosis complex 2; PI3K, phosphoinositide 3-kinase; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IGF-1R, insulin growth factor 1; Akt/PKB, protein kinase B; AMPK, AMP-activated protein kinase; LKB1, liver kinase B1; ILK, integrin-linked kinase.

including activation of the PI3K/Akt/mTOR pathway in vitro.[25] In HER2-positive breast cancer cell lines, trastuzumab inhibited the feedback loop activation of Akt.[26] In addition, lapatinib, a specific dual inhibitor of HER2 and EGFR tyrosine kinase activity, has been shown to induce cell cycle arrest and apoptosis by inhibiting HER2-activated signaling pathways including PI3K/Akt/mTOR.[27]

In cells demonstrating trastuzumab resistance, amplified signaling through both the PI3K/Akt/mTOR and MAPK pathways is evident in the presence of elevated levels of activated receptor tyrosine kinase Eph receptor A2 (EphA2).[28] Involvement of the mTOR pathway in HER2-positive trastuzumab-resistant patients is further supported by preliminary biomarker analyses from the neoadjuvant phase 3 GeparQuattro study (NCT00288002),[29] evaluating the addition of capecitabine and/or trastuzumab (in HER2-positive patients only) to epirubicin/cyclophosphamide followed by docetaxel combination chemotherapy. Increased levels of phosphorylated eIF4E-binding protein (4E-BP1), a substrate of mTOR, have been detected in the HER2-positive cohort of patients resistant to trastuzumab-cytotoxic therapy.[30] This contribution of mTOR to resistance will be further validated in the ongoing phase 3 GeparQuinto study (NCT00567554)[29] investigating the integration of bevacizumab, everolimus, and lapatinib into neoadjuvant chemotherapy regimens.

Since the discovery of the key role of mTOR in the regulation of cell growth and proliferation, cell metabolism, angiogenesis, and apoptosis, two mTOR complexes have been identified: mTORC1, composed of mTOR, regulatory-associated protein of mTOR (RAPTOR), and mTOR-associated protein, LST8 homolog (mLST8 [GβL]), which regulates protein synthesis, cell growth and proliferation, cell metabolism, and angiogenesis; and mTORC2, composed of mTOR, rapamycin-insensitive companion of mTOR (RICTOR), mLST8, and sin1, which phosphorylates the kinase Akt and drives full activation of Akt when combined with pyruvate dehydrogenase kinase isoenzyme 1 (PDK1).[31] New agents are being developed that can inhibit both mTORC1 and mTORC2 and affect the activation of Akt, to limit constitutive downstream mTOR activation (Table 1).[32–39]

A key component of the mTOR pathway is the lipid kinase PI3K, which affects survival, proliferation, growth, shape, migration, and intracellular sorting in tumor development.[40] Three classes of PI3K enzymes, designated I to III, have been identified; members of PI3K class I have been implicated in the mTOR pathway and are a focus of potential therapy for solid tumors.[40] Agents such as BEZ235, PI-103, and XL765 are dual PI3K/mTOR inhibitors, which may bypass feedback loops, potentially increasing their efficacies.[41] These agents are currently being evaluated in clinical trials and may provide targeted therapeutic combinations that are effective against solid tumors.[18,41]

Approved and Investigational mTOR Inhibitors

Several mTOR inhibitors have been developed and evaluated as antitumor therapies, with some distinctive differences in metabolism, formulation, and schedule of administration (Table 1).[32,38,42] Three agents have received United States Food and Drug Administration (FDA) approval (sirolimus, everolimus, and temsirolimus), and two of these (sirolimus and everolimus) are oral agents. Sirolimus (rapamycin), an agent that partially inhibits mTOR,[43] is approved for the prevention of kidney transplant rejection and has been investigated in combination with various agents to determine its clinical activity in breast cancer (Fig. 2A).[29,44,45] Analogs of sirolimus demonstrating more favorable pharmacokinetics include temsirolimus, everolimus, and ridaforolimus (formerly deforolimus) (Fig. 2B–D).[42,46,47] Temsirolimus, approved for the treatment of advanced renal cell carcinoma (RCC),[46] is a prodrug, and its primary active metabolite is rapamycin (half-life approximately 17 to 55 hours).[46] In breast cancer trials, as for RCC, temsirolimus is administered by a weekly intravenous infusion. Everolimus (half-life approximately 30 hours), another analog of sirolimus, is approved for the treatment of advanced RCC and progressive neuroendocrine tumors of pancreatic origin (PNET) at a dosage of 10 mg/day, adults with renal angiomyolipoma and tuberous sclerosis complex (TSC) not requiring immediate surgery at a dosage of 10 mg/day, and subependymal giant cell astrocytomas (SEGAs) associated with tuberous sclerosis at a dosage of 5 mg/day.[47] Everolimus recently (July 2012) received FDA approval for the treatment of postmenopausal women with advanced

Table 1. Approved and investigational mTOR and mTOR combination inhibitors.

Drug	Drug class	Stage of development	Mechanism of action/ binding site
Sirolimus Rapamycin	mTOR inhibitor	Approved 1999 for renal transplant rejection; phase 1/2/3 clinical trials	Binds to FKBP12 and interferes with the FRB domain of mTOR[32]
Temsirolimus CCI779	mTOR inhibitor	Approved 2007 for advanced renal cell carcinoma	Prodrug of rapamycin; binds to FKBP12 and interferes with the FRB domain of mTOR[32]
Everolimus RAD001	mTOR inhibitor	Approved 2009 for advanced renal cell carcinoma after failure with sunitinib or sorafenib; approved 2010 for TS-associated SEGAs requiring therapeutic intervention, but not candidates for curative surgical resection* approved 2011 in PNET that are unresectable, locally advanced or metastatic; approved 2012 for renal angiomyolipoma and TS not requiring immediate surgery and for postmenopausal women with advanced HR+/HER2- breast cancer in combination with exemestane after failure of treatment with letrozole or anastrozole	Binds to FKBP12 and interferes with the FRB domain of mTOR[32]
Ridaforolimus (deforolimus) AP23573	mTOR inhibitor	Phase 3 clinical trials	Binds to FKBP12 and interferes with the FRB domain of mTOR[32]
PI-103	Dual kinase inhibitor	Preclinical	Inhibits mTOR and PI3K[33]
PP242	mTOR inhibitor	Preclinical	Inhibits mTORC1 and mTORC2[34,35]
BN107	mTOR inhibitor	Preclinical	Inhibits mTORC1 and mTORC2[36]
LY294002	Dual kinase inhibitor	Preclinical	Inhibits mTOR and PI3K[35,39]
NVP-BEZ235	Dual kinase inhibitor	Phase 1/2 clinical trials (NCT00620594)[29]	Inhibits PI3K, mTORC1, and mTORC2[33]
SF1126	Dual kinase inhibitor	Phase 1/2	Inhibits mTORC1, mTORC2, and PI3K[37]
XL765	Dual kinase inhibitor	Phase 1	Inhibits mTOR and PI3K[37]
BGT226	Dual kinase inhibitor	Phase 1/2 (NCT00742105; NCT00600275)[29]	Inhibits mTOR and PI3K[38]

Notes: *The effectiveness of everolimus is based on an analysis of change in SEGA volume. Clinical benefit, such as improvement in disease-related symptoms or increase in overall survival, has not been demonstrated.

Abbreviations: HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; HR+, hormone receptor-positive; mTOR, mammalian target of rapamycin; FKBP12, FK506-binding protein; FRB, FKBP-rapamycin-binding; PI3K, phosphoinositide 3-kinase; PNET, progressive neuroendocrine tumors of pancreatic origin; SEGA, subependymal giant cell astrocytoma; TS, tuberous sclerosis.

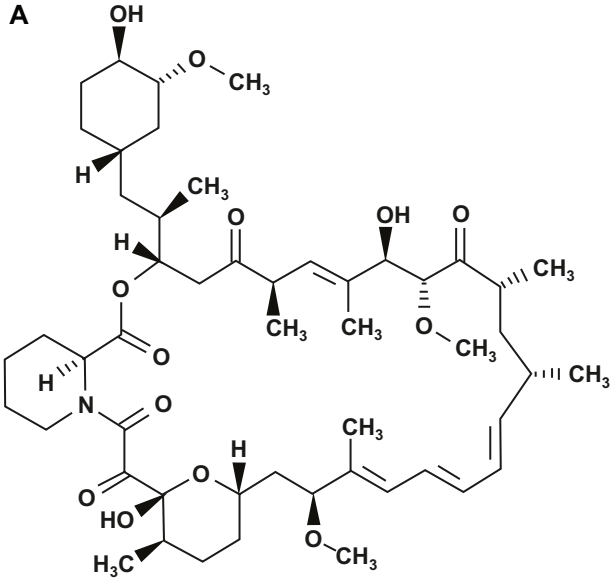

Figure 2A. Sirolimus.[45]
Notes: (3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34, 34a-hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclohentriacontine-1,5,11,28,29 (4H, 6H,31H)-pentone.

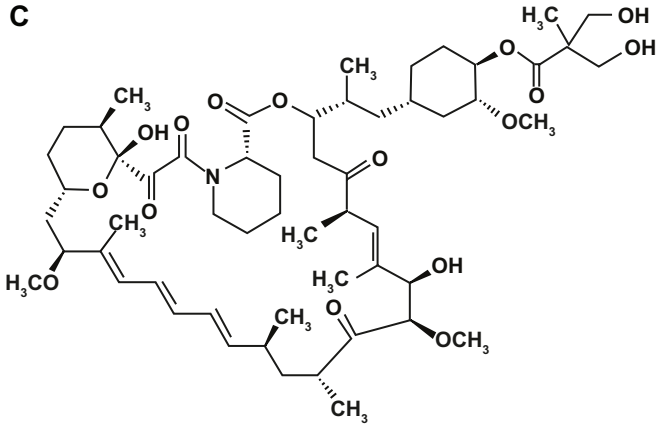

Figure 2C. Temsirolimus.[46]
Notes: (3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclohentriacontine-1,5,11,28,29(4H,6H,31H)-pentone 4′-[2,2-bis(hydroxymethyl)propionate]; or Rapamycin, 42-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate.

hormone receptor-positive, HER2-negative breast cancer in combination with exemestane after failure of treatment with letrozole or anastrozole at a dosage of 10 mg/day.[47] Ridaforolimus remains an investigational analog of sirolimus (half-life 49 hours) that is administered orally in breast cancer trials at 40 mg/day for 5 days/week (NCT01234857, NCT01220570, and NCT00736970).[29,48] Overall, these different mTOR inhibitors share class-specific toxicities such as stomatitis, noninfectious pneumonitis, infection, hyperglycemia, and dyslipidemia.[45–48]

mTOR Inhibition in Combination with Chemotherapy

A variety of chemotherapeutic regimens are used in the treatment of patients with metastatic breast cancer (mBC); however, most tumors eventually develop

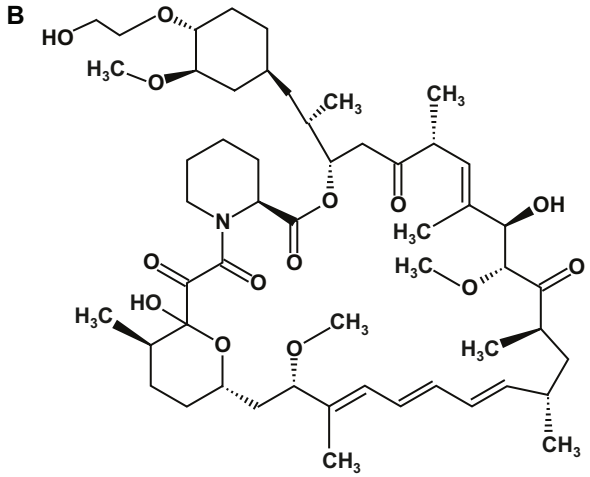

Figure 2B. Everolimus.[47]
Notes: (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.0^{4,9}] hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone.

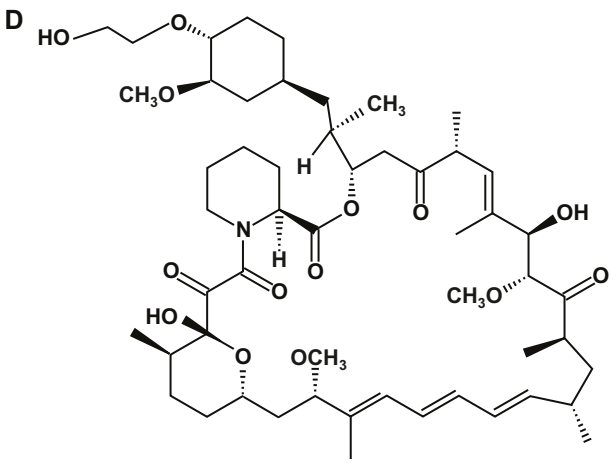

Figure 2D. Ridaforolimus.[42]
Notes: (1R,2R,4S)-4-[(2R)-2-[(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraen-12-yl] propyl]-2-methoxycyclohexyl dimethylphosphinate.

resistance, necessitating a change in treatment. Because the mTOR pathway is upregulated in many chemoresistant cancers, mTOR inhibitors are a logical choice to resensitize tumor cells to chemotherapy or delay the development of treatment resistance by these upregulated or mutated key pathways.

Preclinical evidence

Preclinical studies suggest that one mechanism of chemoresistance to paclitaxel and carboplatin is overexpression of HER2.[49] In breast cancer models with HER2 overexpression, the combination of an mTOR inhibitor with paclitaxel, carboplatin, or vinorelbine enhances apoptosis of the cells in vitro and antitumor efficacy in mouse xenograft models in vivo compared with either agent alone.[49] Moreover, the combination of mTOR inhibitors with cytotoxic agents demonstrates synergistic antiproliferative cellular activity irrespective of HER2 signaling, further suggesting the potential clinical utility of combination therapy for patients with breast cancer that has become resistant to chemotherapy.[49]

Clinical studies in HER2-positive and HER2-negative mBC

A number of nonrandomized studies in HER2-positive trastuzumab-resistant mBC have shown antitumor activity with the addition of everolimus to standard chemotherapy plus trastuzumab (Table 2). Several phase 1 studies have been undertaken to evaluate the tolerability and activity of these combinations. In a phase 1 study of 47 evaluable heavily pretreated HER2-positive mBC patients progressing while receiving trastuzumab and subsequently treated with everolimus plus vinorelbine and trastuzumab, the overall response rate (ORR) was 19%, with a disease control rate of 83% and median progression-free survival (PFS) of 30.7 weeks.[50] The optimal dosing of everolimus in combination with vinorelbine and trastuzumab was determined to be 5 mg/day.[50] In a second, smaller phase 1 study conducted in this same resistant HER2-positive mBC patient population, subsequent treatment with everolimus in combination with paclitaxel plus trastuzumab yielded an ORR of 44%; overall disease

Table 2. Registered combination clinical trials of everolimus in patients with HER2-positive breast cancer.[29]

NCT ID/title	Receptor status	Drug treatments	Phase	N*
NCT00458237/Dose Escalation Followed by Study of Everolimus in Combination with Trastuzumab in HER2-Positive Metastatic Breast Cancer	HER2-positive	Everolimus Trastuzumab	1/2	11
NCT00317720/Trastuzumab and Everolimus in Patients with HER2 Overexpressing Breast Cancer	HER2-positive	Everolimus Trastuzumab	1/2	47
NCT00674414/Trastuzumab with or without Everolimus in Treating Women with Breast Cancer That Can Be Removed by Surgery	HER2-positive	Everolimus Trastuzumab	2	120
NCT00426556/Efficacy and Safety of Everolimus in Combination Therapy, in Patients with HER2-Overexpressing Metastatic Breast Cancer	HER2-positive	Everolimus Paclitaxel Trastuzumab	1/2	90
NCT01163929/A Study to Look at the Combination of Chemotherapy, Trastuzumab and Everolimus in HER2-Positive Breast Cancer	HER2-positive	Everolimus Paclitaxel Trastuzumab	2	30
NCT00876395/BOLERO-1 Everolimus in Combination with Trastuzumab and Paclitaxel in the Treatment of HER2-Positive Locally Advanced or Metastatic Breast Cancer	HER2-positive	Everolimus Trastuzumab Paclitaxel	3	717
NCT01007942/BOLERO-3 Daily Everolimus in Combination with Trastuzumab and Vinorelbine in HER2/Neu Positive Women with Locally Advanced or Metastatic Breast Cancer	HER2-positive	Everolimus Vinorelbine Trastuzumab	3	572
NCT01283789/Lapatinib and RAD001 for HER2 Positive Metastatic Breast Cancer	HER2-positive	Lapatinib RAD001	2	45

Note: *Actual or anticipated enrollment.
Abbreviation: HER2, human epidermal growth factor receptor 2.

was controlled for ≥6 months in 74% of patients, and the median PFS was 34 weeks.[51] The optimal dosing of everolimus in combination with paclitaxel and trastuzumab was determined to be 10 mg/day.[51]

In HER2-negative mBC, everolimus phase 1 combinations have included evaluations with a weekly cisplatin/paclitaxel doublet. In 13 evaluable patients with advanced HER2-negative mBC with a median of 3 previous chemotherapy regimens treated with everolimus and weekly cisplatin/paclitaxel, 1 complete response, 2 partial responses, 7 stable diseases, and 3 disease progressions were noted.[52] In a similar study of HER2-negative, hormone receptor-positive mBC treated with the combination of capecitabine plus everolimus, a nearly identical pattern of response was seen, with 2 partial responses and 7 stable diseases in 12 evaluable patients.[53] Additional studies assessing a variety of everolimus/chemotherapy combinations are ongoing (Table 3).[29]

On the basis of these promising results and those generated from other studies, 24 registered clinical trials—7 of which include HER2-positive patients—are evaluating the efficacy of everolimus in combination with varying chemotherapeutic agents (Tables 2–5)[29] in a variety of disease settings. Two international, double-blind, placebo-controlled phase 3 studies have been initiated to assess the addition of everolimus to trastuzumab-containing chemotherapy regimens in women with HER2-positive locally advanced or mBC (Table 2).[29] In the BOLERO-1 (Breast cancer trials of oraL everolimus) study, 717 patients with untreated metastatic HER2-positive breast cancer have been randomly assigned to receive first-line weekly paclitaxel plus

Table 3. Registered combination clinical trials of everolimus in patients with HER2- and/or triple-negative breast cancer.[29]

NCT ID/title	Receptor status	Drug treatments	Phase	N*
NCT01272141/A Study of Lapatinib in Combination with Everolimus in Patients with Advanced, Triple Negative Breast Cancer	Triple-negative	Everolimus Lapatinib	2	43
NCT00499603/Paclitaxel Followed by 5-Fluorouracil, Epirubicin, Cyclophosphamide (FEC) Versus Paclitaxel and Everolimus Followed by FEC in Women with Breast Cancer	Triple-negative	Everolimus Paclitaxel 5-Fluorouracil Epirubicin Cyclophosphamide	2	50
NCT01031446/Cisplatin, Paclitaxel, and Everolimus in Treating Patients with Metastatic Breast Cancer	Triple-negative HER2-negative	Everolimus Cisplatin Paclitaxel	1/2	55
NCT00915603/Placebo-Controlled Trial of Weekly Paclitaxel/Bevacizumab +/– Everolimus as First-Line Chemotherapy	HER2-negative	Everolimus Paclitaxel Bevacizumab	2	110
NCT00567554/Exploring the Integration of Bevacizumab, Everolimus, and Lapatinib Into Current Neoadjuvant Chemotherapy Regimens for Primary Breast Cancer (GeparQuinto)	HER2-negative or HER2-positive	Everolimus Epirubicin Cyclophosphamide Docetaxel Bevacizumab Paclitaxel Trastuzumab Lapatinib	3	2547
NCT00930930/A Phase II Neo-Adjuvant Study of Cisplatin, Paclitaxel with or without RAD001 in Patients with Triple-negative Locally Advanced Breast Cancer	Triple-negative	Everolimus Cisplatin Paclitaxel Placebo	2	102
NCT00934895/Phase I/II Study of Weekly Abraxane and RAD001 in Women with Locally Advanced or Metastatic Breast Cancer	HER2-negative	Everolimus Albumin-bound Paclitaxel	1/2	72

Note: *Actual or anticipated enrollment.
Abbreviation: HER2, human epidermal growth factor receptor 2.

Table 4. Registered combination clinical trials of everolimus in patients with HER2-negative hormone receptor-positive breast cancer.[29]

NCT ID/title	Hormone status	Drug treatments	Phase	N*
NCT00912340/Randomized Phase II Trial of Trastuzumab or Everolimus in Hormone-refractory Metastatic Breast Cancer	ER-positive and/or PR-positive; HER2-negative	Everolimus Trastuzumab	2	80
NCT00107016/Everolimus and Letrozole as Preoperative Therapy of Primary Breast Cancer in Post-menopausal Women	ER-positive	Everolimus Letrozole	2	255
NCT01298713/Tamoxifen-RAD001 Versus Tamoxifen Alone in Patients with Anti-aromatase Resistant Breast Metastatic Cancer	ER-positive and/or PR-positive; HER2-negative	Everolimus Tamoxifen	2	111
NCT00570921/Study of Combined Fulvestrant and Everolimus in Advanced/Metastatic Breast Cancer After Aromatase Inhibitor Failure	ER-positive and/or PR-positive	Everolimus Fulvestrant	2	44
NCT00863655/BOLERO-2 Everolimus in Combination with Exemestane in the Treatment of Postmenopausal Women with Estrogen Receptor Positive Locally Advanced or Metastatic Breast Cancer Who Are Refractory to Letrozole or Anastrozole	ER-positive HER2-negative	Everolimus Exemestane	3	705
NCT01231659/Safety and Efficacy of Everolimus in Combination with Letrozole in the Treatment of Postmenopausal Women with Locally Advanced or Metastatic Breast Cancer	ER-positive	Everolimus Letrozole	4	70

Note: *Actual or anticipated enrollment.
Abbreviation: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

trastuzumab with or without 10 mg/day of everolimus. The BOLERO-3 study is evaluating 572 patients with HER2-positive mBC previously treated with a taxane and resistant to trastuzumab. These patients have been randomly assigned to receive vinorelbine plus trastuzumab with or without 5 mg/day of everolimus. A third BOLERO trial, BOLERO-2, recruited 724 postmenopausal women with HER2-negative, locally advanced breast cancer or mBC refractory to letrozole or anastrozole who have been randomly assigned to receive exemestane with or without everolimus. In earlier-stage disease, the neoadjuvant GeparQuinto phase 3 European trial evaluated the addition of bevacizumab, everolimus, or lapatinib in 2547 patients with varied hormone status treated with current neoadjuvant chemotherapy regimens (Table 3).[29] Preliminary results did not show a significant clinical advantage for the addition of bevacizumab to standard chemotherapy and demonstrated reduced efficacy with lapatinib versus trastuzumab in combination with epirubicin/cyclophosphamide before surgery.[54] Additionally, recent results from the study arm evaluating the addition of everolimus to paclitaxel in HER2-negative patients after epirubicin/cyclophosphamide/bevacizumab suggest that adding everolimus to paclitaxel did not significantly improve pathological complete response rates compared with paclitaxel alone.[55]

Other mTOR inhibitors also are under evaluation in both HER2-negative and HER2-positive mBC disease settings. One such trial is evaluating

Table 5. Registered combination clinical trials of everolimus in patients with receptor status not specified.[29]

NCT ID/title	Receptor status	Drug treatments	Phase	N*
NCT00930475/Everolimus and Carboplatin in Pretreated Metastatic Breast Cancer	NS	Everolimus Carboplatin	1/2	54
NCT00253318/Everolimus Plus Docetaxel in Patients with Metastatic Breast Cancer	NS	Everolimus Docetaxel	1/2	15

Note: *Actual or anticipated enrollment.
Abbreviation: NS, not specified.

Table 6. Registered combination clinical trials of other mTOR inhibitors in patients with breast cancer.[29]

NCT ID/title	Hormone status	Drug treatments	Phase	N*
NCT00411788/A Phase 2 Study of Rapamycin and Trastuzumab for Patients with HER2 Receptor Positive mBC	HER2-positive	Sirolimus Trastuzumab	2	30
NCT00736970/Oral Deforolimus with Trastuzumab for Patients with HER2+ Trastuzumab-Refractory mBC (8669-009)	HER2-positive	Ridaforolimus Trastuzumab	2	34
NCT01234857 A Study of Ridaforolimus (MK-8669) in Combination with Dalotuzumab (MK-0646) Compared to Standard of Care Treatment in ER+ Breast Cancer Patients (MK-8669-041)	ER-positive; HER2-negative	Ridaforolimus Dalotuzumab Exemestane	2	352
NCT01082068/Study of XL147 or XL765 in Combination with Letrozole in Subjects with Breast Cancer	ER-positive and/or PR-positive HER2-negative	XL765 XL147 Letrozole	1/2	124

Note: *Actual or anticipated enrollment.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; mBC, metastatic breast cancer; PR, progesterone receptor.

sirolimus with trastuzumab in HER2-positive mBC (Table 6).[29] Temsirolimus, an mTOR inhibitor approved for the treatment of advanced RCC, is being evaluated in several mBC trials in combination with chemotherapy as well as with other biologic targeted agents. In patients with locally recurrent or mBC, the combination of cixutumumab, a monoclonal antibody that is an IGF-1R antagonist, and temsirolimus is undergoing evaluation in a phase 1/2 trial to characterize the tolerability and toxicity profile, recommended dose level, and antitumor activity (in terms of ORR) (NCT00699491).[29] As in BOLERO 3, temsirolimus is also being evaluated in combination with vinorelbine ditartrate in patients with unresectable or metastatic solid tumors (NCT01155258).[29] Both locally recurrent or unresctable locally advanced breast cancers in addition to mBC are eligible along with several other tumor types to determine the maximal tolerated dose for this temsirolimus and vinorelbine combination as well as to obtain preliminary information regarding the activity, safety, and tolerability of this combination.[29] Neratinib, a dual inhibitor of HER2 and EGFR kinases, is also being combined with temsirolimus in an open-label, single-arm, dose-escalation phase 1/2 study to determine the maximum tolerated dose, safety, and efficacy of this combination in

Table 7. Registered combination clinical trials of temsirolimus in patients with breast cancer.[29]

NCT ID/title	Receptor status	Drug treatments	Phase	N*
NCT00061971/Letrozole with or without CCI-779 in Treating Postmenopausal Women with Locally Advanced or Metastatic Breast Cancer	ER-positive and/or PR-positive	Temsirolimus Letrozole	2	108
NCT00062751/Study Evaluating Temsirolimus in Breast Neoplasms	ER-positive and/or PR-positive	Temsirolimus Letrozole	2	108
NCT00699491/Cixutumumab and Temsirolimus in Treating Patients with Locally Recurrent or Metastatic Breast Cancer	NS	Temsirolimus Cixutumumab (IMC-A12)	1/2	68
NCT01111825/A Phase 1/2 Trial of Temsirolimus Plus Neratinib for Patients with Metastatic HER2-Amplified or TN Breast Cancer	HER2-positive or triple-negative	Temsirolimus Neratinib (HKI-272)	1/2	65

Note: *Actual or anticipated enrollment.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; NS, not specified; PR, progesterone receptor; TN, triple-negative.

advanced breast cancer (Table 7) (NCT01111825).[29] The phase 1 portion of the study will examine this combination in patients with trastuzumab-refractory HER2-amplified disease or triple-negative disease, whereas the phase 2 portion will study each receptor type separately.[29] Results from these and other studies will help to determine the benefit of temsirolimus combination therapy in advanced breast cancer. Unfortunately, the results of a larger, randomized, phase 3 trial in approximately 900 women with hormone receptor-positive mBC, evaluating first-line treatment with temsirolimus (10 mg/day or 30 mg/day for 5 days every 2 weeks) in combination with the aromatase inhibitor letrozole (2.5 mg/day) versus letrozole (2.5 mg/day) alone, failed to demonstrate clinical benefit.[56] This large trial was closed prematurely, and it has been postulated that the surprising lack of activity has been attributed to both the dose and schedule of temsirolimus as well as the possibility that the first-line mBC disease setting may not be the best setting to assess the impact of mTOR inhibition, which may be more prevalent with later lines of disease as the mTOR signaling pathway may not have been fully activated.[29]

mTOR Inhibition in Combination with HER2-Targeted Therapy

As discussed, trastuzumab with or without an Akt/mTOR pathway inhibitor[25,26] and lapatinib[27] have been shown to exert antitumor effects by inhibiting HER2-activated signaling pathways, including PI3K/Akt/mTOR. A combination of HER2-targeted agents and mTOR inhibitors has demonstrated antitumor activity in resistant breast cancer cells, suggesting that mTOR inhibitors may have the potential to delay or prevent acquired resistance to HER2-targeted agents.[57] Furthermore, PI3K pathway activation due to low expression/loss of function of PTEN and/or mutations in the *PI3KCA* gene has been identified as a major determinant of poor treatment success of trastuzumab- and lapatinib-based therapy in patients with HER2-overexpressing breast cancer.[20,58] Thus, using mTOR inhibitors to restore or enhance trastuzumab sensitivity is a rational approach.

Preclinical evidence

Preclinical studies in HER2-overexpressing mouse tumors and cell lines suggest that PI3K/Akt/mTOR pathway inhibition is required for optimal antitumor effects of HER2 antagonists[57] and may be a clinically applicable strategy for overcoming trastuzumab resistance caused by hyperactivation of the PI3K pathway due to PTEN deficiency.[20,26] In vitro, low doses of everolimus significantly increased growth inhibition by trastuzumab; everolimus also enhanced the antitumor efficacy of trastuzumab in vivo in mouse xenograft models.[26]

Clinical studies

Results of phase 1 studies in patients with HER2-overexpressing mBC resistant to trastuzumab showed that everolimus is well tolerated in combination with trastuzumab and paclitaxel or vinorelbine and that the combination had antitumor activity and formed the basis of the now closed,[50,51] previously mentioned phase 3 trials of everolimus with chemotherapy and trastuzumab in patients with HER2-positive mBC (BOLERO-1 [NCT00876395], BOLERO-3 [NCT01007942]).[29] As noted above, several clinical studies are exploring the potential of everolimus as well as other mTOR inhibitors for improving the efficacy of, or overcoming resistance to, HER2-targeted therapy as outlined above with sirolimus and temsirolimus. These combinations of an mTOR inhibitor with select antimicrotubule agents demonstrate antitumor activity in patients refractory to both trastuzumab and taxanes.[50]

mTOR Inhibition in Combination with Endocrine Therapy

The ER is expressed in approximately 30% of premenopausal patients with breast cancer and 60% to 70% of postmenopausal patients with breast cancer and has a significant role in cancer cell proliferation and metastasis.[59–62] Approximately 30% of patients with ER-positive breast cancer are intrinsically resistant to hormonal therapies, and the remainder of patients eventually acquire resistance to hormonal therapy.[63] In 25% of primary breast cancers, mutations in *PI3 KCA* have been identified and are associated with the development of resistance to therapy.[20] Endocrine resistance has been associated with dysregulated PI3K/Akt/mTOR signaling, and it is hypothesized that cross-talk between the ER pathway and the PI3K/Akt/mTOR pathway underlies this mechanism of resistance to endocrine therapy.[10,64,65]

For example, ER has been shown to activate tyrosine kinase receptors, resulting in the activation of downstream resistance pathways such as ERK/MAPK and PI3K/Akt.[64] It is hypothesized that the addition of an mTOR inhibitor may restore antitumor response to endocrine therapy.[65,66]

Preclinical evidence

In preclinical breast cancer models using MCF-7 cells with constitutively active Akt/mTOR that exhibit hormone and chemotherapy resistance, mTOR inhibitors enhanced the efficacy of selective ER modulators tamoxifen, raloxifene, and ERA-923[67]; the ER downregulator fulvestrant[66]; and the aromatase inhibitor letrozole.[68] In addition, dual inhibition of mTOR and ER signaling in cellular models of breast cancer was shown to have a synergistic effect on cell cycle arrest and induction of apoptosis.[69] The strategy of combination therapy of a selective ER modulator with rapamycin or temsirolimus has also been shown to restore sensitivity to endocrine therapy in breast cancer cells and may be attributed to the return of normal apoptotic response to endocrine therapy.[70] In fact, the BOLERO-2 trial was based on the preclinical combination therapy data with letrozole plus everolimus shown to restore sensitivity to letrozole in breast cancer cells by inhibiting cell cycle progression and triggering apoptotic cell death.[68] Additionally, a phase 1 dose-escalating study that evaluated everolimus plus letrozole in postmenopausal women or men with stable mBC or progression after ≥ 4 months of first- or second-line therapy with letrozole alone found that an everolimus dose of 10 mg/day provided antitumor activity with no pharmacokinetic interactions.[71]

Clinical studies

Several clinical studies have explored and reported results for the potential of mTOR inhibitors to improve efficacy or overcome resistance to endocrine therapy. Recently reported robust results from the international randomized, placebo-controlled, phase 3 study (BOLERO-2, NCT00863655) have generated the most enthusiasm and excitement for the further advancement of mTOR inhibitors in breast cancer. The first of the BOLERO trials evaluating everolimus in mBC, BOLERO-2, evaluated the combination of everolimus and exemestane in patients with ER-positive mBC refractory to letrozole or anastrozole, demonstrating that the addition of everolimus to exemestane significantly improved PFS (Table 4).[29] In an interim analysis, median PFS was 6.9 months for everolimus plus exemestane compared with 2.8 months for exemestane plus placebo, corresponding to a 57% reduction in the hazard ratio (HR = 0.43, 95% confidence interval [CI], 0.35–0.54, $P < 0.001$ [local assessment]).[72] A longer-term prespecified 12-month follow-up analysis of BOLERO-2 was conducted, with results confirming the benefit reported with shorter follow-up with a median PFS now at 7.4 months versus 3.2 months for exemestane only (HR = 0.44, 95% CI, 0.36–0.53, $P < 0.001$ [local assessment]).[73] Furthermore, the median dose intensity (cumulative dose/duration of exposure) of everolimus was 8.6 mg (range, 0.3–10 mg) and the benefit of PFS was maintained in patients whose dose intensity during the study was lower possibly due to adverse events (AEs) (<7.5 mg/day, HR = 0.40, 95% CI, 0.31–0.52; ≥7.5 mg/day, HR = 0.45, 95% CI, 0.37–0.56).[74] Even longer-term data, at 18 months' follow-up, everolimus plus exemestane improved PFS versus exemestane alone. There was an estimated 55% risk reduction for PFS (HR = 0.45; 95% CI, 0.38–0.54; $P < 0.001$) corresponding to a clinically meaningful 4.63-month prolongation in median PFS, from 3.19 to 7.82 months.[75] The AEs observed in the BOLERO-2 trial are consistent with those reported with everolimus and other rapamycin analogs and include stomatitis, fatigue and asthenia, diarrhea, cough, pyrexia, and hyperglycemia.[56,76] The most common grade 3 or 4 AEs in the everolimus plus exemestane and exemestane-only groups were stomatitis (8% vs. 1%, respectively), anemia (6% vs. <1%, respectively), dyspnea (4% vs. 1%, respectively), hyperglycemia (4% vs. <1%, respectively), fatigue (4% vs. 1%, respectively), and pneumonitis (3% vs. 0, respectively).[72]

Positive results for TAMRAD, a randomized phase 2 trial of 111 patients investigating the combination of tamoxifen and everolimus in patients with hormone receptor-positive or progesterone receptor-positive and HER2-negative mBC previously treated with an aromatase inhibitor and any numbers of previous chemotherapies, were recently reported.[77] In the intent-to-treat analysis, the clinical benefit rate in the tamoxifen and everolimus group was 61.1% (95% CI,

46.9–74.1) versus 42.1% (95% CI, 29.1–55.9) in the tamoxifen-only group, yielding a clinically significant difference (P = 0.045). With a 2-year median follow-up, the time to progression was increased from 4.5 months in the tamoxifen-only group to 8.6 months in the tamoxifen and everolimus group. The HR of progression was 0.54 (95% CI, 0.36–0.81, P = 0.002 [exploratory]). Furthermore, the probability of survival was 54% in the tamoxifen and everolimus group and 30% in the tamoxifen-only group (HR = 0.45, 95% CI, 0.24–0.81, P = 0.007).[77] The safety profile of tamoxifen and everolimus was similar to that seen in other studies examining everolimus. Stomatitis, rash, diarrhea, anorexia, and infection were more common in the tamoxifen and everolimus group than in the tamoxifen-only group. Most AEs were graded 1 or 2 in severity and could be managed without stopping everolimus. Grade 3 or 4 AEs were not more common in tamoxifen-everolimus recipients than in tamoxifen-only recipients, further supporting the acceptable safety profile of tamoxifen-everolimus therapy.[77] In addition to pain, the most common nonhematologic AEs in the tamoxifen-everolimus group compared with the tamoxifen-alone group were fatigue (72% vs. 53%, respectively), stomatitis (56% vs. 7%, respectively), rash (44% vs. 7%, respectively), anorexia (43% vs. 18%, respectively), and diarrhea (39% vs. 11%, respectively).[77] The most common hematologic AEs in the tamoxifen-everolimus group compared with the tamoxifen-alone group were decreased hemoglobin levels (69% vs. 35%, respectively) and decreased lymphocyte (48% vs. 21%, respectively) and leukocyte (54% vs. 18%, respectively) counts.[77]

mTOR inhibition, in combination with the selective ER downregulator fulvestrant, has also demonstrated promising activity in patients with metastatic ER-positive breast cancer in which aromatase inhibitors were not effective. Interim results from this ongoing, single-arm, phase 2 study demonstrated a median time to progression of 8.6 months (of 11 evaluable patients, 7 had stable disease and 1 had partial response).[78]

In earlier-stage disease, letrozole plus everolimus or letrozole plus placebo was recently tested in a randomized, phase 2, neoadjuvant trial in 270 postmenopausal women with ER-positive breast cancer. The everolimus combination resulted in improved tumor response rate compared with letrozole/placebo (68% vs. 59%, respectively, P = 0.06), as well as in a greater antiproliferative response (assessed by Ki67 expression) than placebo (NCT00107016).[79] Preclinical data together with these positive study results support future and ongoing clinical trials with mTOR inhibitors in mBC.

mTOR Inhibition in Combination with Other Signal Transduction Inhibitors

Another potential strategy is to combine mTOR inhibitors with other inhibitors of signal transduction pathways. The IGF-1 pathway is a major contributor to breast cancer pathogenesis. IGF-1R and the insulin receptor substrate 1 frequently are expressed at increased levels in breast cancers and are associated with chemoresistance in breast cancer.[21,80] mTOR integrates incoming signals from insulin or IGF via receptor-mediated activation of the PI3K/mTOR/Akt signaling pathway.[16] An explanation for the limited activity of mTOR inhibitor monotherapy in breast cancer is that mTOR inhibition blocks negative feedback on IGF-1R signaling, impinging on PI3K and resulting in an increase in PI3K and Akt activation, which counteracts the inhibition of mTOR.[81] Under normal conditions, IGF-1R–induced activation of the mTOR pathway elicits a negative feedback loop whereby mTOR/S6 kinase reduces insulin receptor substrate I levels, the main ligand for the IGF-1R.[82] mTOR inhibition interferes with feedback regulation of upstream signaling elements, thereby paradoxically activating IGF-I signaling and lessening the antitumor effectiveness of mTOR inhibition. Treatment with the mTOR inhibitor rapamycin results in increased Akt activity and Akt phosphorylation at S473, which is dependent on IGF-1R and is associated with increased levels of insulin receptor substrate-1 (IRS-1).[82] Along these lines, blockade of IGF-1 and its receptor, IGF-1R, enhanced the antiproliferative effect of rapamycin, and, therefore, blocking IGF-1R appears to prevent mTOR inhibitor–induced feedback activation of PI3K/Akt and sensitizes tumor cell lines (including breast cancer) to the antiproliferative effects of mTOR inhibitors.[82] As such, this preclinical evidence suggests that IGF-1R inhibitors restore sensitivity in breast cancer cells that are resistant to mTOR inhibition. Currently, the combination of mTOR inhibitors and IGF-1R inhibitors is under study in several clinical trials, such as in the phase 1 trial evaluating temsirolimus in combination with cixutumumab (IMC-A12) against IGF-1R

mentioned previously (Table 7) (NCT00699491).[29] Similarly, treatment with weekly doses (50 to 70 mg) of RAD001 (everolimus) resulted in activation of the ERK/MAPK pathway in tumor biopsies from patients with metastatic cancer.[83] Inhibition of MAPK (via upstream inhibition of ERK with a MEK1/2 inhibitor) enhanced the antitumor action of rapamycin.[83] In both instances, sensitivity to mTOR inhibitors was enhanced by a combination of agents that target multiple aspects of the PI3K/Akt/mTOR pathway. The clinical utility of mTOR inhibitors may be limited by complex signaling feedback loops. Therefore, more clinical studies are needed with combined therapeutic approaches aimed at the mTOR pathway.

Other Investigational mTOR Inhibitors in Breast Cancer

Additional novel inhibitors of the PI3K/Akt/mTOR pathway are being assessed in early-phase clinical trials. BEZ235, an investigational dual PI3K/mTOR inhibitor, has shown additive effects with trastuzumab and lapatinib in a PTEN knockdown breast cancer cell line.[84] Additionally, BEZ235 has demonstrated antitumor activity in trastuzumab-resistant breast cancer cells with activating mutations in p110α, in trastuzumab-resistant breast cancer xenografts,[85] and in lapatinib-resistant breast cancer cells in vitro.[86] This drug currently is being tested in phase 1 clinical trials of patients with mBC and other solid tumors alone (NCT00620594)[29] and in combination with other agents (NCT01285466, NCT01248494).[29]

Ridaforolimus, an investigational oral mTOR inhibitor, improved PFS compared with placebo in patients with metastatic soft tissue or bone sarcomas who previously had a favorable response to chemotherapy in the SUCCEED trial (NCT00538239).[29,87] In October 2011, the FDA accepted the new drug application filing for ridaforolimus in the treatment of metastatic soft tissue or bone sarcoma in patients who had a favorable response to chemotherapy. The use of ridaforolimus in mBC in combination with the IGF-1R inhibitor is currently being explored in a phase 2 study in HER2-negative, ER-positive, high-proliferation breast cancer.[29]

Conclusions

The PI3K/Akt/mTOR pathway contributes to estrogen independence, growth factor independence, and the development of resistance not only to chemotherapy but also to endocrine and biologic targeted HER2 agents in advanced breast cancer. Inhibitors to mTOR have demonstrated antitumor activity in a variety of cancer types, including hormone receptor–positive, HER2-negative advanced breast cancer, RCC, PNET, renal angiomyolipoma associated with TSC and SEGA associated with TSC, for which they have gained FDA approval. BOLERO-2 has demonstrated that the combination of mTOR inhibitors with endocrine therapies is a promising strategy, with the mTOR inhibitor everolimus, in combination with exemestane, improving PFS in patients with hormone receptor–positive advanced breast cancer refractory to nonsteroidal aromatase inhibitors. Furthermore, the strategy of combining mTOR inhibitors with other targeted agents or with cytotoxic chemotherapy has produced signals of antitumor activity or has delayed the development of resistance to these agents. Selective patient criteria and rational selection of combination therapies may enhance the success of mTOR therapies based on information emerging from the many ongoing clinical trials of mTOR combinations in mBC. Designing effective combination therapies using mTOR inhibitors together with different agents that target key molecular elements involved in breast cancer relies heavily on the identification of predictive markers that may provide a basis for individualized patient therapy. Biomarker-based evaluations in ongoing and future trials may identify the most beneficial therapeutic strategies for different patient subgroups. Future clinical studies may stratify patients based on previous responsiveness or resistance to endocrine or trastuzumab therapy. Although mTOR inhibitors already play a critically important role in delivering more effective cancer therapy, their role as partners to current treatment strategies for advanced breast cancer continues to be defined.

Author Contributions

Conceived and designed the experiments: DAY. Analyzed the data: DAY. Wrote the first draft of the manuscript: DAY. Contributed to the writing of the manuscript: DAY. Agree with manuscript results and conclusions: DAY. Jointly developed the structure and arguments for the paper: DAY. Made critical revisions and approved final version: DAY. The author DAY reviewed and approved of the final manuscript.

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Competing Interests

DY is a consultant to Novartis.

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