REVIEW-THEMED ISSUE

The therapeutic potential of mTOR inhibitors in breast cancer

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Rapamycin and modified rapamycins (rapalogs) have been used to prevent allograft rejection after organ transplant for over 15 years. The mechanistic target of rapamycin (mTOR) has been determined to be a key component of the mTORC1 complex which consists of the serine/threonine kinase TOR and at least five other proteins which are involved in regulating its activity. Some of the best characterized substrates of mTORC1 are proteins which are key kinases involved in the regulation of cell growth (e.g., p70S6K) and protein translation (e.g., 4E-BP1). These proteins may in some cases serve as indicators to sensitivity to rapamycin-related therapies. Dysregulation of mTORC1 activity frequently occurs due to mutations at, or amplifications of, upstream growth factor receptors (e.g., human epidermal growth factor receptor-2, HER2) as well as kinases (e.g., PI3K) and phosphatases (e.g., PTEN) critical in the regulation of cell growth. More recently, it has been shown that certain rapalogs may enhance the effectiveness of hormonal-based therapies for breast cancer patients who have become resistant to endocrine therapy. The combined treatment of certain rapalogs (e.g., everolimus) and aromatase inhibitors (e.g., exemestane) has been approved by the United States Food and Drug Administration (US FDA) and other drug regulatory agencies to treat estrogen receptor positive (ER+) breast cancer patients who have become resistant to hormonal-based therapies and have progressed. This review will summarize recent basic and clinical research in the area and evaluate potential novel therapeutic approaches.

Introduction

It has been shown that the PI3K/PTEN/Akt/mTORC1 kinase cascade is frequently expressed aberrantly in breast and other cancers, which can result in abnormal proliferation. One clinical method to suppress the activity of this cascade is to inhibit the mTOR kinase, a key component present in the mTORC1 complex in this pathway. One approved drug family which suppresses mTOR is the rapamycin family (as known as rapalogs). Rapamycin was originally observed and prescribed to prevent allograft rejection in organ transplant patients. Now rapamycin and certain rapalogs are being used to treat various cancer patients, including breast cancer patients.

Breast cancer is one of the most frequent malignancies in women as approximately one in eight women will develop breast cancer in their lifetime. In the United States, this corresponds to approximately 231 840 new cases of invasive breast cancer each year, which will result in approximately 40 290 deaths [1].

One of the recent major advances in breast cancer therapy is the discovery that certain breast cancers which remain hormone receptor positive (HR+) but have become resistant to endocrine therapy can be treated by the modified rapamycin, everolimus. Everolimus (AFINITOR®), in combination with the aromatase inhibitor exemestane (AROMASIN®), was approved by the US FDA in 2012 to treat HR+, HER2− breast

cancer. Everolimus targets the mTORC1 complex, which is often dysregulated in breast cancers that have become resistant to hormone-based therapies. Suppression of mTORC1 results in inhibition of p70S6K and other proteins. The expression of these proteins is dysregulated in breast cancer patient samples as compared to normal tissues. p70S6K expression is associated with a poor prognosis in breast cancer [2].

Rapamycin and rapalogs used in treatment of human diseases

Rapalogs are synthetic drugs which are analogues of rapamycin. Rapamycin was originally purified from the bacterium *Steptomyces hygroscopicus*. The rapalogs have better pharmacokinetic kinetic properties than the natural rapamycin. mTORC1 blockers (rapalogs) have been evaluated in clinical trials with breast and other cancer patients [3–7]. Interestingly, different rapalogs have displayed different effects. For example, temsirolimus (TORISEL®) did not show any benefits when compared with endocrine therapy alone. Sirolimus (RAPAMUNE®, rapamycin) has shown promising results in phase II clinical trials [7].

Rapamycin (sirolimus, RAPAMUNE®) was approved by the US FDA to prevent rejection in kidney transplant patients. Temsirolimus (TORISEL®) was approved by the US FDA and European Medicines Agency (EMA) for the treatment of renal cell carcinoma in 2007. Everolimus (AFINITOR®) was approved for the treatment of advanced kidney cancer in 2009 and for prevention of organ transplantation rejection after renal transplant in 2010. In addition, it was approved for treatment of subependymal giant cell astrocytoma (SEGA) that is associated with tuberous sclerosis (TS) in 2010 in those patients who cannot have surgical intervention. It was also approved in 2011 for treatment of patients containing inoperable or metastatic neuroendocrine tumours of pancreatic origin. Everolimus has been approved by the USA FDA for the prevention of organ rejection in liver transplant patients since 2013. Since 2015, sirolimus has been approved by the US FDA for the treatment of lymphangioleiomyomatosis (LAM). LAM is a rare disease affecting lungs, kidneys and the lymphatic system and is progressive.

The rapalogs temsirolimus (TORISEL®) and everolimus (AFINITOR®) are being used to treat breast and renal cancer patients [7–9]. The identification of markers which could predict response to therapy is important. Furthermore, the effects of a combination of these rapalogs with other anti-cancer drugs are being evaluated.

Endocrine therapy-aromatase inhibitors (AIs)

Commonly used non-steroidal AIs include letrozole (FEMARA®) and arimidex (ANASTROZOLE®). These non-steroidal AIs inhibit estrogen synthesis by a reversible competition for aromatase.

Exemestane (AROMASIN®) is an oral steroidal AI. It binds irreversibly to aromatase and inhibits its activity. It prevents the conversion of cholesterol into pregnenolone thereby suppressing the conversion of androgenic precursors into estrogens. AI therapy has been used in the treatment of all stages (0 to IV) of breast cancer. AI therapy has been used to prevent metastasis of estrogen receptor positive (ER+) breast cancers. AI therapies have been associated with musculoskeletal problems and hot flashes [9]. A diagram illustrating the site of action of certain estrogen-receptor antagonists and AIs and their effects on ERα-mediated gene expression is presented in Figure 1.

Endocrine therapy estrogen-receptor antagonists

4-hydroxyl-tamoxifen (4HT, NOLVADEX®) binds to the ER and competes with estrogen. 4HT belongs to the group of compounds which are called selective estrogen-receptor modulators (SERMS) (Figure 1). Other SERMS include raloxifene and toremifene. SERMS accelerate the proteasomal degradation of ERα. Fulvestrant (FASLODEX®) is a pure estrogen receptor antagonist. Exemestane treatment is superior to tamoxifen treatment as documented in a phase III clinical trial [10]. The superior effects of AIs in comparison to tamoxifen, especially in combination with rapalogs such as everolimus, have been summarized recently [7–10].

Markers of sensitivity to therapy

The expression and localization of p70S6K1, p70S6K2 and phosphorylated (activated) p70S6K (T389) were examined in two large breast cancer studies. p70S6K1 was associated with high proliferation, HER2 expression and cytoplasmic Akt expression. p70S6K2 was associated with ER+, low proliferation and nuclear Akt (S473) expression. The expression of phosphorylated p70S6K (T389) was correlated with a low benefit to treatment with tamoxifen. The cytoplasmic *vs.* nuclear localization of p70S6K was determined to be related to benefit with tamoxifen as nuclear p70S6K was associated with reduced benefit to tamoxifen. In contrast, cytoplasmic p70S6K was associated with a significant benefit to tamoxifen. High p70S6K1 protein expression was associated with a poor prognosis whether it was located in the nucleus or cytoplasm. Thus, p70S6K expression and localization could be a marker to predict those patients which may benefit from tamoxifen [11].

Combining estrogen-receptor antagonists and rapalogs in breast cancer therapy

Approximately 75% of breast cancer patients are HR+ and may be initially sensitive to hormonal-based therapies which target the HR. The development of resistance to hormonal-based therapies is a significant problem in breast cancer treatment. The PI3K/PTEN/Akt/mTORC1 pathway is frequently hyper-activated in endocrine-resistant breast cancer.

A link between ERα and mTORC1 has recently been discovered. mTORC1 can phosphorylate ERα on S167 via p70S6K which results in its activation. mTORC1 can phosphorylate ERα on S104/S106 which leads to the transcription of ERα-target genes [12].

The results of a randomized phase II trial of the combination of tamoxifen and everolimus in patients with HER2−, metastatic breast cancer that had previously been treated with AI in the TAMRAD study were presented. The TAMRAD study was a randomized, noncomparative phase II study. The TAMRAD study attempted to determine the efficacy and safety of tamoxifen in combination with everolimus in AI-resistant metastatic breast cancer patients. The TAMRAD study revealed that the combination of tamoxifen and everolimus increased the clinical benefit rate (CBR) from 42% with tamoxifen alone to 61% with everolimus and tamoxifen. The time to progression (TTP)
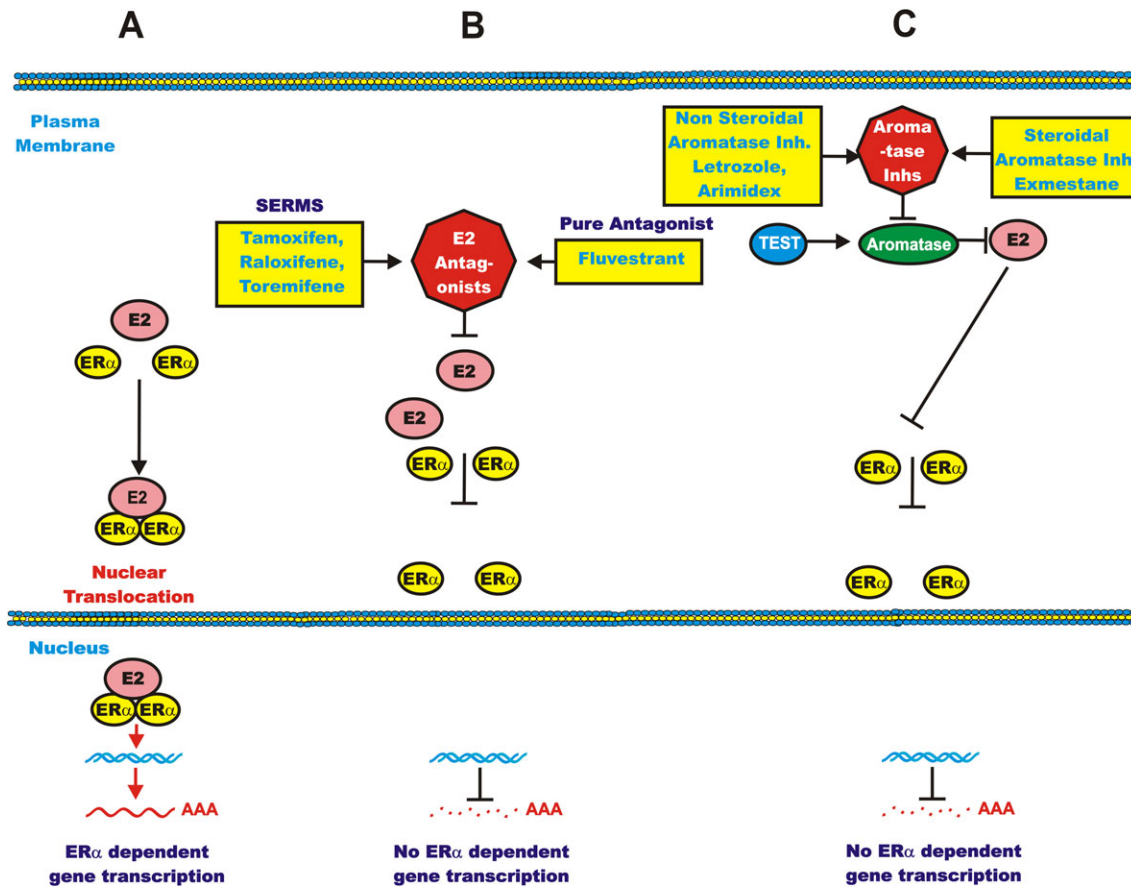
Figure 1

Effects of estrogen, estrogen receptor antagonists and aromatase inhibitors on ERα-mediated signalling: (A) normal ERα-mediated signalling; (B) estrogen receptor antagonists block ERα signalling; (C) lack of conversion of testosterone (TEST) to estrogen by aromatase inhibitors preventing conversion. SERMs = selective estrogen-receptor modulators

increased from 4.5 months with tamoxifen alone to 8.6 months in those patients treated with tamoxifen and everolimus. The risk of death was reduced by 55% upon treatment with tamoxifen and everolimus *vs.* tamoxifen alone [13]. A letter to the editor commented on the appropriateness of the study design in this clinical trial [14]. The TAMRAD trial showed benefits to those patients who initially responded to AIs but subsequently became resistant. However, the combination did not appear to be beneficial for patients with *de novo*, previously untreated metastatic HR+ breast cancer [15].

Clinical samples were examined from 52 patients in the TAMRAD trial. Subgroups were identified based on TTP and examined for various markers. The tamoxifen/everolimus-treated samples with the most improvement in TTP compared with the tamoxifen-only treated subgroup patients were determined to express high levels of p4EBP1, low levels of 4EBP1, LKB1, pAkt and PI3K. In 45 patient samples examined for mutation at *PIK3CA* and *KRAS*, 20% had mutations at *PIK3CA* and one patient (2%) had a mutation at *KRAS*. In this study, a positive correlation between downstream targets of mTORC1 and PI3K/PTEN/Akt/mTORC1 activation and everolimus efficiency was observed. The authors pointed out that a larger number of patient samples should be examined [15].

The BRE-43 study examined the effects of a combination of everolimus and the complete ER antagonist fulvestrant (FASLODEX®) in ER+ patients with metastatic breast cancer. The phase II trial examined this drug combination with postmenopausal woman that were previously treated with an AI who exhibited disease progression or relapse. It was determined that everolimus plus fulvestrant was effective in AI-pretreated ER+ breast cancer patients whose cancer had metastasized. However, not all patients benefited from this therapeutic approach. The discovery of additional biomarkers may allow the selection of patients who will benefit [16].

Combining aromatase inhibitors and rapalogs in breast cancer therapy

The effects of everolimus has been examined in combination with the nonsteroidal AI letrozole (FEMARA®) in metastatic ER + breast cancer patients in a phase II randomized study. The addition of everolimus to letrozole increased the response rate from 59.1 to 68.1%. Downregulation of phosphoribosomal protein S6 (rpS6) was detected in the everolimus-treated patients [17].

The effects of combining letrozole with temsirolimus have been examined in a phase III clinical trial as a first line

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endocrine therapy in postmenopausal women who had locally advanced or metastatic breast cancer [18]. This study revealed that adding temsirolimus to letrozole did not appear to improve the progression-free survival (PFS) in this group of AI-naïve breast cancer patients.

Everolimus in combination with exemestane for HR+, HER2− metastatic breast cancer patients

The Breast Cancer Trials of Oral Everolimus-2 (BOLERO-2) study evaluated the effects of combining everolimus and exemestane for endocrine-resistant HR+, HER2− breast cancer [19]. The FDA approved the combination of everolimus and exemestane to treat HR+, HER2− breast cancer patients who progressed on treatment with letrozole or anastrazole (Arimidex®, a nonsteroidal AI) AI treatments. The everolimus and exemestane combined treatment resulted in better therapeutic outcomes [19]. The primary end point in the BOLERO-2 clinical trial was PFS and indicated that the combination of everolimus and exemestane improved by more than two-fold PFS in HR+, HER2− breast cancer patients that had progressed after treatment with nonsteroidal AIs. In contrast, analysis of the secondary end point, which was overall survival (OS), did not result in a statistically significant difference (Clinical Trial #NCT00863655) [20].

The BOLERO-2 clinical trial also revealed that treatment with everolimus and exemestane had positive effects on bone marrow turnover and progressive disease in bone [21].

Inflammation of the mucous membrane of the mouth (stomatitis) is the most frequent side effect associated with everolimus treatment. If stomatitis is severe, it may result in either discontinuation of everolimus treatment or methods to relieve the stomatitis (steroid-based mouth rinses) [22].

A summary of a meta-analysis of randomized breast cancer clinical trials with patients treated with AIs with or without everolimus has been published [23]. The analysis was obtained from six studies which included 3693 women who were treated with everolimus plus exemestane or placebo plus exemestane. Everolimus plus exemestane increased the overall response rate (ORR), relative risk, PFS and clinical benefit compared to placebo control as well as the adverse side effects compared to the placebo control (stomatitis, rash, hyperglycaemia, diarrhoea, anorexia and pneumonitis).

Everolimus in combination with letrozole as frontline therapy for HR+, HER2− metastatic breast cancer patients

The Breast Cancer Trials of Oral Everolimus-4 (BOLERO-4) study is a phase II clinical trial determining the effects of everolimus plus letrozole as a first-line therapy for HR+, HER2− metastatic breast cancer patients [24]. This trial will evaluate whether there is a role for continuing everolimus and AI treatment after progression. This trial will also examine whether the combination of everolimus plus letrozole can be used after progression in those breast cancer patients treated with everolimus plus exemestane [25].

Activation of the PI3K/PTEN/AKT/MTOR pathway in endocrine-resistant breast cancer

The PI3K/PTEN/AKT/mTORC pathway is frequently dysregulated in many different cancer types including: breast [26, 27], pancreatic [28, 29], prostate [30], brain [31, 32], leukaemia [33, 34] and others [35–38]. This pathway is regulated at many steps by tumour suppressor and oncogenes [35, 36]. Mutations can occur at multiple steps in this pathway [37, 38]. In some cases dysregulation of this signalling pathway can result in drug and therapeutic resistance [39]. The mutation rates of components of this and other pathways in breast and other cancers can be found at The Cancer Atlas Data Base: https://tcga-data.nci.nih.gov/tcga/. There are many different mechanisms which can result in alteration of the activity of the PI3K pathway. Activating mutations at many different genes can occur (e.g., *PIK3CA*), while mutations which silence tumour suppressor genes can occur (e.g., *PTEN*). In addition, epigenetic mechanisms as well as miRNAs and lncRNAs can alter the activity of this pathway [40, 41]. A diagram illustrating where some of the mutations that occur in signalling pathways is presented in Figure 2.

Targeting the PI3K/PTEN/Akt/mTORC1 pathway is a very active area of basic and clinical research as well as patient therapy [42, 43]. Various components of this pathway have been targeted including PI3K [44], PTEN [45, 46], Akt [47], mTORC1 [44] as well as critical downstream components including GSK-3 [37, 38] and NF-κB [48]. A diagram illustrating the PI3K/PTEN/Akt/mTORC1 pathway and some sites where inhibitors target key components is presented in Figure 3.

Tamoxifen will inhibit the growth of bulk adherent breast cancer cells (BC) but not the breast cancer stem cells (CSCs). Tamoxifen-treated CSCs were determined to express proteins involved in the PI3K/PTEN/Akt/mTORC1 pathway including proteins associated with ribosome biogenesis and mRNA translation including p70S6K1, rpS6 and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). Combined treatment of the breast CSCs with tamoxifen and rapamycin or the rapalog everolimus or the dual PI3K/mTOR inhibitor PF-04691502 suppressed mammosphere formation. These studies indicate the importance of the mTORC1 pathway in mammosphere formation and endocrine resistance [49].

Everolimus in combination with other drugs to treat herceptin-resistant breast cancer patients

Activation of the PI3K/PTEN/Akt/mTORC1 pathway has been shown to be involved in herceptin resistance in HER2+ breast cancers. A phase II safety and clinical activity trial with oral sirolimus, in combination with herceptin (trastuzumab), was performed with patients with metastatic breast cancer who exhibited disease progression after prior herceptin therapy. The combination of sirolimus and herceptin appeared to be well tolerated in this study. There was also some disease activity. Thus, in some HER-resistant tumours, mTOR inhibition may overcome herceptin resistance [50].

It was known that rapalogs would suppress herceptin resistance in *PTEN*-deficient tumours and make the normally herceptin-resistant tumours sensitive to herceptin. The BOLERO-1 clinical trial (ClinicalTrials.gov, number NCT00876395) examined the safety of combining everolimus and herceptin and paclitaxel in the treatment of advanced HER2+ breast cancer patients. The advanced breast cancer patients enrolled in this
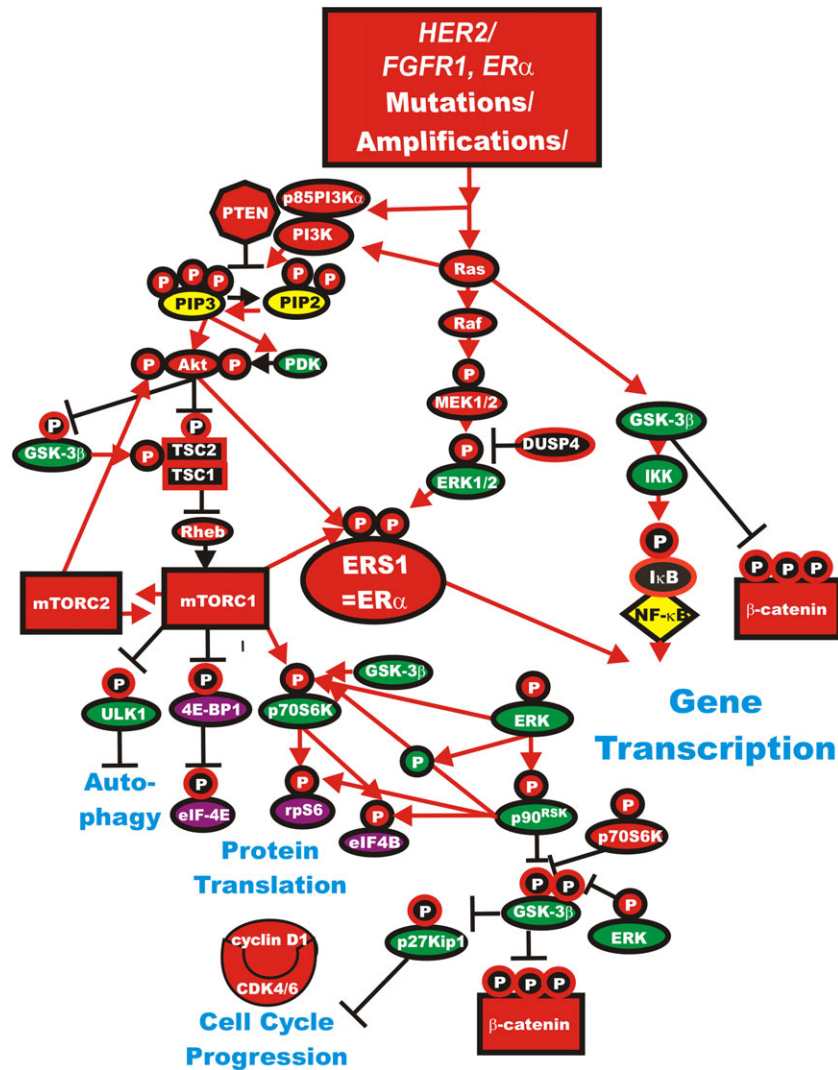
Figure 2

Genetic mutations which result in activation of the Ras/PI3K/PTEN/Akt/mTORC1 and Ras/Raf/MEK/ERK signalling pathways and contribute to malignant transformation and therapy resistance. Sometimes dysregulated expression of growth factor receptors occurs by genetic mutations, translocations or genomic amplifications which can lead to activation of the Ras/Raf/MEK/ERK, Ras/PI3K/PTEN/Akt/mTORC1 and other signalling pathways. Genes in the Ras/PI3K/PTEN/Akt/mTORC1 and Ras/Raf/MEK/ERK pathways that have activating mutations detected in human cancer and proliferative diseases are indicated in red ovals and squares. Other key genes are indicated in green ovals. Red arrows indicate activating events in pathways. Blocked black arrows indicating inactivating events in pathways

study had not been treated with either herceptin or chemotherapy within 12 months of randomization. This study revealed that the PFS was not significantly different between the full analysis population (everolimus and herceptin and paclitaxel *vs.* placebo and herceptin and paclitaxel). Although this study did not meet its prespecified criteria for significance, the authors reported that the HR-/HER2+ population treated with everolimus needs further evaluation as in this subgroup, a 7.2-month prolongation was observed in the arm that received everolimus and herceptin and paclitaxel in comparison to placebo and herceptin and paclitaxel [51].

The BOLERO-3 phase III clinical trial examined the effects of everolimus in combination with other drugs (vinorelbine,

NAVELBINE®) in HER2+, herceptin-resistant breast cancer patients. Addition of everolimus to the combination of herceptin plus vinorelbine prolonged PFS in advanced herceptin-resistant breast cancer patients who had previously been treated with taxanes (ClinicalTrials.gov number NCT01007942) [52]. While the BOLERO-3 trial was statistically significant, the clinical usefulness may be limited and did not lead to an indication in this breast cancer subtype.

Use of rapalogs to overcome herceptin resistance in HER2+ breast cancers

In a RNA interference study of 8000 genes, only *PTEN* suppression was determined to be responsible for herceptin resistance
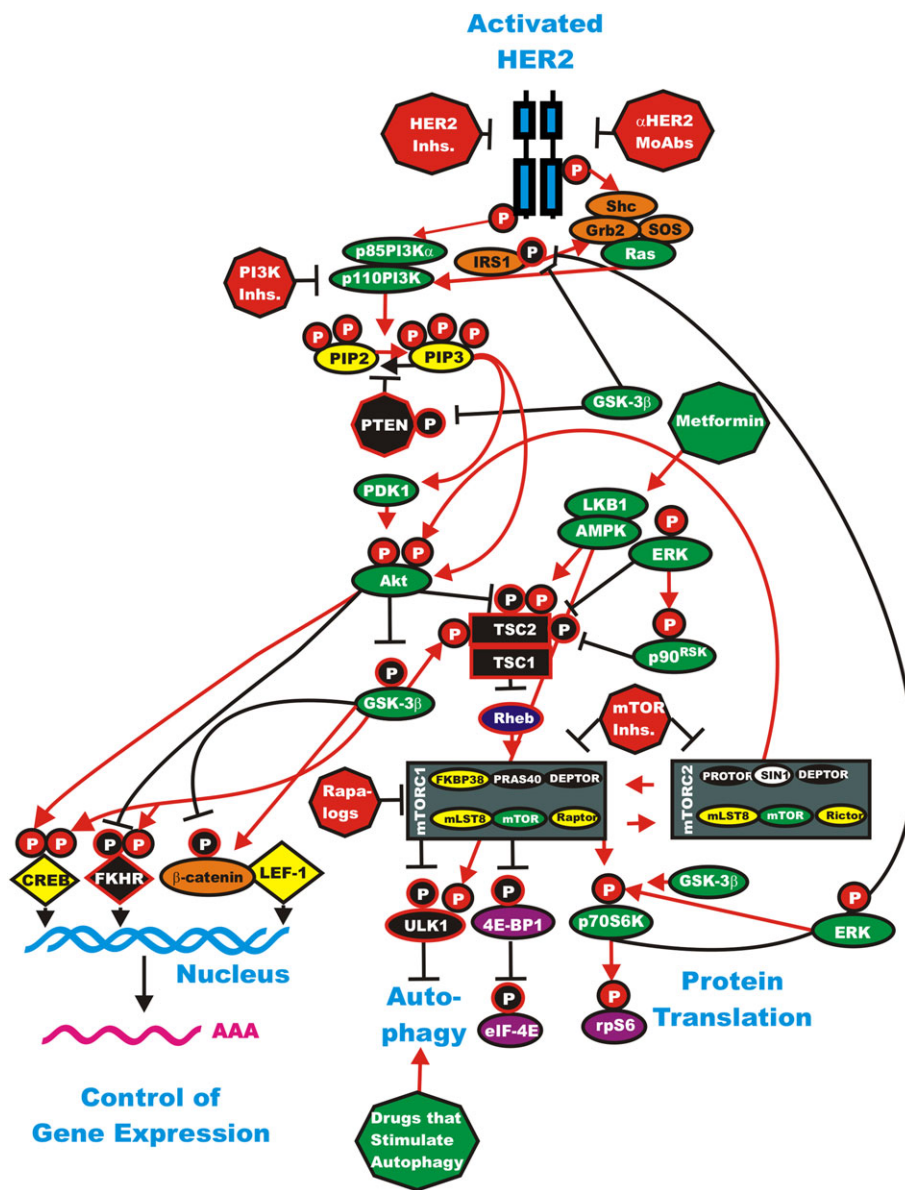
Figure 3

Overview of the HER2/PI3K/PTEN/Akt/mTORC pathway and potential sites for intervention with small molecule membrane-permeable inhibitors and monoclonal antibodies (MoAbs). The HER2 receptor is indicated in blue. In this figure it is depicted as a homodimer, although it can also heterodimerize with other EGFR family members. The downstream PI3K/PTEN/Akt/mTORC1 pathway is regulated by Ras (indicated in green ovals), PTEN indicated in a black octagon, insulin regulated substrate 1 (IRS1) Shc, Grb2, Sos and β-catenin are indicated in orange ovals. Kinases are indicated in green ovals. The p85 regulatory subunit of PI3K (p85PI3Kα) is indicated in a green oval. The phosphatases which inhibit steps in this pathway are indicated in black octagons. TSC1 and TSC2 are indicated in black squares. PIP2 and PIP3 are indicated in yellow ovals. The mTORC1 blockers (rapalogs), PI3K and mTOR inhibitors are indicated in red octagons. The AMPK activator metformin is indicated in a green octagon. mTOR interacting proteins, which positively regulate mTOR activity, are indicated in yellow ovals. mTOR interacting proteins which negatively regulate mTOR activity are indicated in black ovals. Transcription factors activated by either ERK or Akt phosphorylation are indicated in yellow diamonds. The FKHR transcription factor that is inactivated by Akt phosphorylation is indicated by a black diamond and a white P in a black circle. FKHR is also activated by GSK-3β phosphorylation which is indicated by a white P in a red circle. mRNA initiation factors and proteins associated with the ribosome are indicated in magenta ovals. mTORC1 phosphorylates the unc-51-like kinase 1 (ULK1) which results in the suppression of autophagy. ULK1 is indicated in a black oval. In contrast, AMPK activates both ULK1 and autophagy as well as TSC activity. Proteins involved in the regulation of translation are indicated in purple ovals. Red arrows indicate activating events in pathways. Black arrows indicate inactivating events in pathways. Activating phosphorylation events are depicted in red circles with Ps with a black outlined circle. Inactivating phosphorylation events are depicted in black circles with Ps with a red outlined circle

in cells which overexpressed HER2 [53]. Moreover, this study determined that *PTEN*-deficient tumours had lower responses to herceptin than *PTEN*-positive tumours. In subsequent studies,

it was demonstrated that activation of the PI3K/PTEN/Akt/mTOR pathway was associated with shorter PFS in herceptin-treated patients [54]. When everolimus was combined with

Table 1

Breast cancer clinical trials with rapalogs alone or in combination with EGFR/HER2/IGFR inhibitors or hormonal-based agents (to April 2016)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
Rapalogs*						
A randomized study of mTOR inhibition by RAD001 (everolimus) in invasive breast cancer patients after pre-operative use of anthracycline and/or taxane-based chemotherapy	NCT01088893	II	BC	Unknown because information has not been verified recently	Everolimus	Not provided
A phase II trial of short-term everolimus (RAD001) to predict response in women with operable breast cancer	NCT00855114	II	BC	Withdrawn	Everolimus (mTORC1 blocker)	Not provided
Phase II trial of CCI-779 (temsirolimus) in patients with locally advanced or metastatic breast cancer	NCT00376688	II	Male BC, recurrent BC, stage IIIA, stage IIIB, stage IIIC, stage IV BC	Ongoing, but not recruiting	Temsirolimus (mTORC1 blocker)	Not provided
A phase II trial of RAD001 in triple negative metastatic breast cancer	NCT00827567	II	Breast cancer	Terminated	RAD001 (everolimus)	[79–81]
Randomized, double blind, multicentre phase III trial evaluating the safety and benefit of adding everolimus to adjuvant hormone therapy in women with poor prognosis, ER+ and HER2 – primary breast cancer who remain free of disease after receiving 3 years of adjuvant hormone therapy	NCT01805271	III	ER+, HER2 – BC	Recruiting	Everolimus	Not provided
RADAR: a randomized discontinuation phase II study to determine the efficacy of RAD001 in breast cancer patients with bone metastases	NCT00466102	II	BC	Ongoing, but not recruiting	RAD001	Not provided
A randomized phase II study of two different schedules of RAD001C in patients with recurrent/metastatic breast cancer	NCT00255788	II	BC	Completed	Everolimus	Not provided

(continues)

Table 1

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
Rapalogs plus herceptin[†]						
A brief dose escalation followed by a phase II study of RAD001 in combination with trastuzumab in HER2-positive metastatic breast cancer	NCT00458237	I/II	BC	Ongoing, but not recruiting	Everolimus, trastuzumab	[82]
A Phase Ib/II Study Investigating the Combination of Everolimus With Trastuzumab and Paclitaxel in Patients With HER2-overexpressing Metastatic Breast Cancer	NCT00426556	I	Metastatic BC	Completed	Everolimus, trastuzumab, paclitaxel	Not provided
A phase Ib study investigating the combination of everolimus with trastuzumab and vinorelbine in patients with HER2-overexpressing metastatic breast cancer	NCT00426530	I	Breast neoplasms, neoplasm metastasis	Completed	Everolimus, trastuzumab, vinorelbine	[83]
A phase II study of rapamycin (rapamune, sirolimus) and trastuzumab (herceptin) for patients with HER-2 receptor positive metastatic breast cancer	NCT00411788	II	BC	Recruitment is unknown	Rapamycin, trastuzumab	Not provided
A phase II study evaluating the efficacy and tolerability of everolimus (RAD001) In combination with trastuzumab and vinorelbine in the treatment of progressive HER2-positive breast cancer brain metastases	NCT01305941	II	HER2+ BC	Recruiting	Everolimus, vinorelbine, trastuzumab	Not provided
A randomized phase III, double-blind, placebo-controlled multicenter trial of everolimus in combination with trastuzumab and paclitaxel, as first line therapy in women with HER2-positive locally advanced or metastatic breast cancer	NCT00876395	III	BC	Ongoing, not recruiting	Everolimus, trastuzumab, paclitaxel	[51]
A phase II neoadjuvant study of RAD001 (everolimus) in	NCT01163929	II	BC	Withdrawn prior to enrolment	Paclitaxel	Not provided

(continues)

Table 1

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
combination with paclitaxel and trastuzumab for operable HER2-positive breast cancer						
A phase II study evaluating the efficacy and tolerability of everolimus (RAD001) in combination with trastuzumab and vinorelbine in the treatment of progressive HER2-positive breast cancer brain metastases	NCT01305941	II	HER2+ BC	Recruiting	Everolimus, vinorelbine, trastuzumab	Not provided
Phase I-II study of trastuzumab in combination with RAD001 in patients with HER-2 overexpressing, PTEN-deficient metastatic breast cancer progressing on trastuzumab-based therapy	NCT00317720	I/II	BC metastatic	Completed	Trastuzumab, RAD001	[82]
A phase II, randomized, multicentre study, assessing value of adding everolimus (RAD001) to trastuzumab as preoperative therapy of HER-2 positive primary breast cancer amenable to surgery	NCT00674414	II	BC	Terminated due to accrual issue (82 points accrued/120 expected)	Trastuzumab, everolimus	Not provided
Randomized phase II trial of trastuzumab or everolimus in hormone-refractory metastatic breast cancer	NCT00912340	II	BC	Ongoing, not recruiting	Trastuzumab (herceptin), everolimus	Not provided
A randomized phase III, double-blind, placebo-controlled multicentre trial of daily everolimus in combination with trastuzumab and vinorelbine, in pretreated women with HER2/Neu overexpressing locally advanced or metastatic breast cancer	NCT01007942	III	HER2/Neu overexpressing locally advanced BC, metastatic BC	Completed	Everolimus, vinorelbine, trastuzumab	[84]
A phase II trial of oral deforolimus (AP23573; MK-8669), an mTOR inhibitor, in	NCT00736970	II	BC	Completed	Ridaforolimus, trastuzumab	[85]

(continues)

Table 1
(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
combination with trastuzumab for patients with HER2-positive trastuzumab-refractory metastatic breast cancer						
Rapalogs plus EGFR/HER2 or IGF-1R inhibitors[‡]						
Phase II trial of lapatinib and RAD001 for HER2-positive metastatic breast cancer	NCT01283789	II	Metastatic BC	Ongoing not recruiting	Lapatinib, RAD-001	Not provided
Phase I study of combined temosirolimus, erlotinib and cisplatin in advanced solid tumours	NCT00998036	I	TNBC	Completed	Temsirolimus, cisplatin, erlotinib	Not provided
*A phase III trials programme exploring the integration of bevacizumab, everolimus (RAD001), and lapatinib into current neoadjuvant chemotherapy regimes for primary breast cancer	NCT00567554	III	BC	Completed	Epirubicin, cyclophosphamide, docetaxel, bevacizumab, paclitaxel, everolimus, trastuzumab, lapatinib	[86–88]
Phase I/II trial of an oral mTOR protein kinase inhibitor (everolimus, RAD001) in combination with an oral EGFR tyrosine kinase inhibitor (erlotinib, Tarceva™) in patients with metastatic breast cancer	NCT00574366	I	BC	Completed	Erlotinib (EGFR1 inhibitor), everolimus (RAD001)	Not provided
*Phase Ib/II single-arm trial evaluating the combination of lapatinib, everolimus and capecitabine for the treatment of patients with HER2-positive metastatic breast cancer with CNS progression after trastuzumab	NCT01783756	I/II	Central nervous system metastases HER2+ BC, male BC, recurrent BC, stage IV BC	Currently recruiting	Lapatinib ditosylate, everolimus, capecitabine	Not provided
Phase I/II trial of IMC-A12 in combination with temsirolimus in patients with metastatic breast cancer	NCT00699491	I/II	Male BC, recurrent BC, stage IV BC	Ongoing, but not recruiting	Cixutumumab (an IGF-1R inhibitor), temsirolimus	Not provided
	NCT01272141	II	BC	Terminated		Not provided

(continues)

Table 1

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
Phase II trial of lapatinib in combination with everolimus in triple negative metastatic or locally advanced breast cancer					Lapatinib, everolimus	
GCC 0901 – a phase II study of letrozole in combination with lapatinib followed by an addition of everolimus in postmenopausal women with advanced endocrine resistant breast cancer	NCT01499160	II	Breast neoplasms, endocrine breast diseases, neoplasm metastasis	Ongoing, not recruiting	Letrozole, lapatinib, everolimus	Not provided
Rapalogs plus hormonal-based therapies[§]						
A multicentre randomized, double blind, placebo-controlled, phase II study to compare endocrine treatment alone vs. endocrine treatment with everolimus in patients with HR+/HER2 – metastatic breast cancer and progression after previous treatment with exemestane and everolimus	NCT01773460	III	Metastatic BC	Terminated	Everolimus	Not provided
*Phase II study of everolimus in combination with exemestane vs. everolimus alone vs. capecitabine in the treatment of postmenopausal women with ER + locally advanced, recurrent, or metastatic breast cancer after recurrence or progression on prior letrozole or anastrozole	NCT01783444	II	BC	Ongoing, not recruiting	Capecitabine (a pro-drug, which is converted to 5-flurouracil (5-FU) which inhibits thymidylate synthase, exemestane, everolimus	Not provided
A phase Ib/II trial of LEE011 in combination with everolimus (RAD001) and exemestane in the treatment of postmenopausal women with ER+, HER2 – locally advanced or metastatic breast cancer	NCT01857193	I/II	BC	Currently recruiting	LEE011, a CDK4/6 inhibitor, exemestane, everolimus	Not provided
A phase IV multicentre, open label study of postmenopausal women	NCT01743560	IV	ER+ advanced BC	Ongoing, but not recruiting	RAD001, exemestane	Not provided

(continues)

Table 1

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
with ER+ locally advanced or metastatic breast cancer treated with everolimus (RAD001) in combination with exemestane, with exploratory epigenetic marker analysis						
An open-label, phase II, single-arm study of everolimus in combination with letrozole in the treatment of postmenopausal women with ER+ HER2 negative metastatic or locally advanced breast cancer	NCT01698918	II	ER+ BC	Ongoing, not recruiting	Everolimus, letrozole, exemestane	Not provided
*Phase II study of everolimus in combination with exemestane vs. everolimus alone vs. capecitabine in the treatment of postmenopausal women with ER+ locally advanced, recurrent, or metastatic breast cancer after recurrence or progression on prior letrozole or anastrozole	NCT01783444	II	BC	Ongoing, not recruiting	Capecitabine, exemestane, everolimus	Not provided
A phase IIIB, multicentre, open label study for postmenopausal women with ER+ locally advanced or metastatic breast cancer treated with everolimus (RAD001) in combination with exemestane: 4EVER – efficacy, safety, health economics, translational research	NCT01626222	III	Metastatic BC	Completed	Exemestane, everolimus	Not provided
A phase II study of combined fulvestrant (faslodex) and RAD001 (everolimus) in advanced/metastatic breast cancer after aromatase inhibitor failure	NCT00570921	II	BC	Completed	RAD001, fulvestrant	[16]
Circulating FGF21 levels and efficacy of exemestane, everolimus and metformin in postmenopausal women	NCT01627067	II	BC	Ongoing, not recruiting	Everolimus, exemestane, metformin	Not provided

(continues)

Table 1

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
with HR+ metastatic breast cancer and BMI ≥ 25						
A randomized double-blind, placebo-controlled study of everolimus in combination with exemestane in the treatment of postmenopausal women with ER+ locally advanced or metastatic breast cancer who are refractory to letrozole or anastrozole	NCT00863655	III	BC	Completed	Everolimus, exemestane	[19, 89–93]
Phase II open label study of everolimus (RAD001) in combination with letrozole in the treatment of postmenopausal women with locally advanced or metastatic breast cancer women with ER+ after failure of tamoxifen and or anestrozole or examestane	NCT01231659	IV	Postmenopausal women, locally advanced, or metastatic BC	Ongoing, but not recruiting	Everolimus, letrozole	Not provided
Randomized, double-blind, placebo-controlled phase II trial of fulvestrant (faslodex) plus everolimus in postmenopausal patients with HR+ metastatic breast cancer resistant to aromatase inhibitor therapy	NCT01797120	II	Metastatic BC	Ongoing, not recruiting	Fulvestrant, everolimus	No results posted yet
Phase III randomized, placebo-controlled clinical trial evaluating the use of adjuvant endocrine therapy ± one year of everolimus in patients with high-risk, HR+ and HER2/Neu negative breast cancer	NCT01674140	III	BC	Recruiting	Anastrozole, everolimus, exemestane, goserelin acetate, letrozole, leuprolide acetate, tamoxifen citrate	Not provided
A phase II, double-blind, randomized, placebo-controlled, multicentre study assessing the value of adding everolimus to letrozole as preoperative therapy of primary breast cancer in postmenopausal women	NCT00107016	II	BC	Completed	RAD001, letrozole	[17]
A phase II randomized open-label study of	NCT00062751	II	BC	Completed	Letrozole, temsirolimus (CCI-779)	Not provided

(continues)

Table 1

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
letrozole in combination with two dose levels and schedules of oral temsirolimus (CCI-779), or letrozole alone, in postmenopausal women with locally advanced or metastatic breast cancer						
Phase II study assessing the tolerance and efficacy of tamoxifen alone vs. the association tamoxifen-RAD001 (everolimus) in patients with anti-aromatase resistant breast metastatic cancer	NCT01298713	II	BC mTOR proteins	Ongoing, not recruiting	Tamoxifen, everolimus	[15]
A phase II randomized trial of the combination of ridaforolimus and exemestane, compared to ridaforolimus, dalotuzumab and exemestane in high proliferation, ER+ breast cancer patients	NCT01605396	II	BC	Ongoing, but not recruiting	Ridaforolimus, dalotuzumab, exemestane	Not provided
GCC 0901 – a phase II study of letrozole in combination with lapatinib followed by an addition of everolimus in postmenopausal women with advanced endocrine resistant breast cancer	NCT01499160	II	Breast neoplasms, endocrine breast diseases, neoplasm metastasis	Ongoing, not recruiting	Letrozole, lapatinib, everolimus	Not provided
A phase II randomized open-label study of letrozole in combination with two dose levels and schedules of oral temsirolimus (CCI-779), or letrozole alone, in postmenopausal women with locally advanced or metastatic breast cancer	NCT00061971	II	BC	Completed	Letrozole, temsirolimus (CCI-779)	Not provided
A phase III randomized, placebo-controlled, double-blind study of oral CCI-779 administered in combination with letrozole vs. letrozole alone as first line hormonal therapy in postmenopausal women	NCT00083993	III	Breast neoplasms, neoplasm metastasis	Terminated	Temsirolimus (CCI-779) for 34 months, letrozole for 34 months	[18]

(continues)

Table 1
(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
with locally advanced or metastatic breast cancer						
A two-part adaptive, randomized trial of ridaforolimus in combination with dalotuzumab compared to exemestane or compared to ridaforolimus or dalotuzumab monotherapy in ER+ breast cancer patients	NCT01234857	II	BC	Completed	Ridaforolimus, dalotuzumab, exemestane	Not provided

*Everolimus (RAD001, AFINITOR®), Temsirolimus (CCI-779, TORISEL®). †Everolimus (RAD001), Temsirolimus (CCI-779), Deforolimus (Ridaforolimus, AP23573, MK-8669) and Trastuzumab (Herceptin). ‡Everolimus (RAD001),Temsirolimus (CCI-779) and EGFR/HER2 Inhibitor Lapatinib [TYKERB®], EGFR inhibitor Erlotinib [TARCEVA®] and IGF-R1 Inhibitors (IMC-A12, Cixutumumab). §Everolimus (RAD001), Ridaforolimus (Deforolimus), Temsirolimus (CCI-779), and Hormonal Therapy (Exemestane [AROMASIN®], Fulvestrant [FASLODEX®], Letrozole [FEMARA®].

chemotherapy, sensitivity to herceptin was observed in breast cancer models which overexpressed HER2 [55].

Combining everolimus and chemotherapeutic drugs to treat triple negative breast cancer (TNBC)

The effects of combining everolimus and chemotherapeutic drugs (e.g., carboplatin) have been examined in a clinical trial with metastatic TNBC. The trial with everolimus and carboplatin demonstrated efficacy in PFS for 3 months. In this study, clinical benefit was defined as (complete remission (CR) + partial remission (PR) + stable disease (SD) lasting ≥ 6 months). This trial demonstrated a clinical benefit of 36% (ClinicalTrials.gov NCT01127763) [56].

The effects of combining everolimus with neoadjuvant chemotherapy with paclitaxel for 12 weeks followed by 5-flurouracil, epirubicin and cyclophosphamide every three weeks for four cycles was evaluated in patients with TNBC. In this study, no significant differences were observed between the everolimus-treated and the nontreated groups [57].

Markers of sensitivity to rapamycin/rapalogs

A total of 302 tumour specimens from HR+, HER2− patient samples from participants of the BOLERO-2 clinical trial were examined by next-generation sequencing for genetic mutations. Interestingly, it was determined that the benefit from everolimus on PFS was present regardless of genetic alterations in *PIK3CA*, *FGFR1* and *CCND1* or dysregulation of the signalling pathways of which they are components. However, differences in everolimus benefit were observed in *PI3KCA* exon specific mutations (exon 20 *vs.* exon 9) as well as the degree of chromosomal instability [58].

Detection of single nucleotide polymorphism (SNP) signatures may be an approach to find patients which show activation of mTOR. Such signatures may define patients who are sensitive to rapalog treatment [59].

Combining everolimus with metformin

The effects of combining everolimus with the adenosine 5′-monophosphate-activated protein kinase (AMPK) activator metformin have been evaluated. The site of action of metformin is shown in Figure 3. This combination was determined to inhibit the growth and mammosphere formation better than either drug by itself. The combination of everolimus and metformin also suppressed the phosphorylation of downstream targets including rpS6 and 4E-BP1 in HCC1429 cells. The combination of everolimus and metformin was also determined to suppress the development of xenografts better than treatment with either drug by itself [60]. There is a clinical trial examining the effects of everolimus and metformin in breast cancer therapy (NCT01627067).

Combining everolimus with PI3K and TOR inhibitors

Recently, it has become apparent that blocking signalling pathways at multiple levels may be more effective than blocking a pathway at a single position. This may result from multiple mechanisms including intricate negative and positive feedback pathways as well as mutations at different components. TOR inhibitors actually block the kinase activity of

Table 2

Breast cancer clinical trials with rapalogs and chemotherapy or small molecule kinase inhibitors or metformin (up to April 2016)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
Rapalogs and chemotherapy*						
Phase II trial of RAD001 plus carboplatin in patients with triple-negative metastatic breast cancer	NCT01127763	II	BC	Completed	RAD001, carboplatin	[56]
A randomized study of mTOR inhibition by RAD001 (everolimus) in invasive breast cancer patients after pre-operative use of anthracycline and/or taxane-based chemotherapy	NCT01088893	II	BC	Recruitment unknown because the information has not been verified recently	Everolimus	Not provided
Phase I, open label, dose escalation study of the safety, tolerability, and pharmacokinetics of the combination RAD001 plus docetaxel in patients with metastatic breast cancer	NCT00253318	I	BC	Terminated	Docetaxel, RAD001, dexamethasone, a glucocorticoid/ steroidal drugs that has anti-inflammatory, immunosuppressant properties	Not provided
Phase II, open label, dose escalation study of the safety, tolerability, and pharmacokinetics of the combination RAD001 plus docetaxel in patients with metastatic breast cancer	NCT01825265	II	BC	Withdrawn prior to enrolment	Docetaxel, RAD001, dexamethasone	Not provided
Phase I/II study of weekly abraxane and RAD001 in women with locally advanced or metastatic breast cancer. A study of the Cancer Institute of New Jersey Oncology Group (CINJOG)	NCT00934895	I/II	BC	Ongoing, not recruiting	Everolimus, abraxane. Abraxane is paclitaxel bonded to albumin as a delivery vehicle	Not provided
A phase Ib/II study of cisplatin, paclitaxel, and RAD001 in patients with metastatic breast cancer	NCT01031446	I/II	BC	Completed	Cisplatin, everolimus, paclitaxel	Not provided
Everolimus (RAD001) in combination with intravenous carboplatin in taxane- and anthracycline-pretreated patients with progressive metastatic breast cancer	NCT00930475	I/II	BC	Unknown	RAD001 in combination with carboplatin. Carboplatin is related to cisplatin, but is modified	Not provided
Open label randomized clinical trial of standard neoadjuvant chemotherapy (paclitaxel followed by FEC) vs. the combination of paclitaxel and RAD001 followed by FEC in women with triple	NCT00499603	II	BC	Completed	Paclitaxel, 5-FU, epirubicin, cyclophosphamide, RAD001	Not provided

(continues)

Table 2

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
receptor-negative breast cancer (CRAD001C24101)						
A phase I study of cisplatin, paclitaxel, and RAD001 patients with metastatic breast cancer	NCT00680758	I	BC	Completed	Cisplatin, everolimus, paclitaxel	Not provided
A phase II neo-adjuvant study of cisplatin, paclitaxel with or without RAD001 in patients with triple-negative locally advanced breast cancer	NCT00930930	II	BC	Completed	Cisplatin, everolimus, paclitaxel	Not provided
A phase I pilot study of the oral mTOR inhibitor RAD001 in combination with capecitabine for metastatic breast cancer	NCT00473005	I	BC	Terminated due to principal investigator leaving sponsor	Capecitabine, RAD001	Not provided
Phase I study of combined temsirolimus, erlotinib and cisplatin in advanced solid tumours	NCT00998036	I	TNBC	Completed	Temsirolimus, cisplatin, erlotinib	Not provided
A phase Ib study of combination of temsirolimus (Torisel®) and pegylated liposomal doxorubicin (PLD, Doxil® /Caelyx®) in advanced or recurrent breast, endometrial and ovarian cancer	NCT00982631	I	Advanced/recurrent BC, endometrial cancer, ovarian cancer	Recruitment is unknown because the information has not been verified recently	Temsirolimus/PLD	Not provided
A phase I study of the mTOR inhibitor rapamycin (rapamune, sirolimus) in combination with abraxane (paclitaxel protein-bound particles) in advanced solid cancers	NCT00337376	I	Advanced solid cancers	Terminated	Rapamune, abraxane	Not provided
Phase I study of docetaxel and temsirolimus in resistant solid malignancies	NCT00703625	I	Resistant solid malignancies	Completed	Temsirolimus, docetaxel	Not provided
Randomized phase II study to compare vinorelbine in combination with the mTOR inhibitor everolimus vs. vinorelbin monotherapy for second-line treatment in advanced breast cancer	NCT01520103	II	HER2 – metastatic BC, HER2 – locally advanced BC	Recruiting	Vinorebine, everolimus	Not provided
Phase I clinical trial of temsirolimus and vinorelbine in advanced solid tumours	NCT01155258	I	Male BC,	Ongoing, but not recruiting	Temsirolimus, vinorelbine ditartrate	Not provided

(continues)

Table 2

(Continued)

Official trial name	Clinical trial #	Phase of trial	Type of cancer patient in trial	Status of trial	Intervention	Publications
Neoadjuvant phase II study of everolimus plus cisplatin in triple negative breast cancer patients with residual disease after standard chemotherapy	NCT01931163	II	BC, TNBC	Recruiting	Everolimus	Not provided
Everolimus and PI3K or PI3K/mTOR inhibitors†						
A phase I study of BKM120 and everolimus in advanced solid malignancies	NCT01470209	I	Solid tumours	Recruiting participants	BKM120, everolimus	Not provided
An open-label, multicenter phase I dose-finding study of RAD001 (everolimus, Afinitor®) in combination with BEZ235 in patients with advanced solid tumours	NCT01482156	I	Advanced solid tumours, metastatic BC, metastatic renal cell carcinoma	Completed	RAD001 + BEZ235, a dual inhibitor of PI3K and mTOR	Not provided
Rapalogs and Akt inhibitors‡						
Phase I parallel protocol of MK-8669 (ridaforolimus) + MK-2206 and MK-8669 (ridaforolimus) + MK-0752 doublets (MK-MK) in patients with advanced cancer	NCT01295632	I	Advanced cancer	Completed	Ridaforolimus, MK-0752 (gamma secretase inhibitor, a notch signalling pathway inhibitor). MK-2206	[94, 95]
Rapalogs and metformin§						
A phase I study of temsirolimus in combination with metformin in advanced solid tumours	NCT00659568	I	BC, endometrial cancer, kidney cancer, lung cancer, lymphoma, unspecified adult solid tumour, protocol specific	Completed	Metformin hydrochloride, temsirolimus	Not provided
Circulating FGF21 levels and efficacy of exemestane, everolimus and metformin in postmenopausal women with HR+ metastatic breast cancer and BMI ≥ 25	NCT01627067	II	BC	Ongoing, but not recruiting participants	Everloimus, exemestane, metformin	Not provided

*Everolimus (RAD001, Afinitor®), Temsirolimus (CCI-779, Torisel®), Sirolimus (Rapamycin, Rapamune Sirolimus), Ridaforolimus (Deforolimus, AP23573, MK-8669) and Chemotherapy (Abraxane, Anthracycline, Capecitabine, Carboplatin, Cisplatin, Cyclophosphamide, Docetaxel, Epirubicin, 5-Flurouracil, Paclitaxel, Taxane, Vinorelbin). †PI3K (BKM120, Buparlisib), PI3K/mTOR (BEZ235, Dactolisib). ‡Ridaforolimus and Akt Inhibitors (MK-2206). §Everolimus, Temsirolimus and Metformin.

TOR, specifically the TOR serine/threonine kinase that is present in both mTORC1 and mTORC2. Rapalogs block the activity of mTORC1 but not normally mTORC2. There are also dual PI3K and TOR inhibitors which block both PI3K and TOR. The effects of combining the mTORC1 blocker everolimus with PI3K and TOR kinase inhibitors has been examined in a panel of 30 breast cancer cell lines. A correlation between everolimus IC_{50} values and p70S6K phosphorylation was observed in these studies. In contrast, a correlation between everolimus IC_{50} values and Akt or ERK phosphorylation was not observed. The effects of combining everolimus and the kinase inhibitors were also examined on four everolimus-resistant cell lines and inhibition of proliferation was observed. Thus, it may be possible to enhance the effects of everolimus with mTOR and PI3K inhibitors on certain breast cancers [61].

Resistance to rapamycin

In some breast cancer models, activated Akt signalling is associated with rapamycin resistance. This may be due to phosphorylation of the transcriptional repressor 4E-BP1. While rapamycin or the Akt inhibitor MK2206 solo treatment did not have significant effects on suppressing 4E-BP1 phosphorylation, growth or mobility of tumour cells, the combined treatment did have benefits both *in vitro* and *in vivo*. The combined treatment suppressed the phosphorylation of the proline-rich Akt substrate of 40 kDa (PRAS40) on S183 and T246 which are normally mediated by mTORC1 and Akt respectively. This resulted in enhanced binding of dephosphorylated PRAS40 to Raptor/mTOR which repressed mTORC1-mediated 4E-BP1 phosphorylation and protein translation. These studies provide a mechanism of how rapamycin and Akt inhibitors may enhance suppression of tumour growth [62].

The epithelial mesenchymal transition (EMT) has been proposed to confer rapamycin resistance. The epithelial protein E-cadherin was observed to be expressed at higher levels in rapamycin-sensitive cells. In contrast, E-cadherin was expressed at lower levels in mesenchymal breast cancer cells that were less sensitive to rapamycin. MCF-7 breast cancer cells transfected with the constitutively active Snail transcription factor were resistant to rapamycin. Inhibition of ZEB1 transcription factor by transfection of ZEB siRNA in mesenchymal breast cancer cells promoted mesenchymal to epithelial transition (MET), E-cadherin expression and sensitivity to rapamycin. The effects of rapamycin on cell growth could be enhanced by treatment of the mesenchymal cells with the MEK inhibitor trametinib [63].

Resveratrol is a stilbenoid (natural phenol) found in the skin of red grapes, blueberries, raspberries, mulberries and other plants. Resveratrol is induced when the plant is attacked by bacteria or fungi. The effects of rapamycin and resveratrol on the induction of apoptosis and autophagy have been examined in breast cancer cells. Breast cancer cells may often become resistant to rapamycin treatment by up-regulation of the Ras/PI3K/PTEN/Akt/mTORC1 pathway due to the induction of autophagy which results in the prevention of apoptosis. Addition of rapamycin and resveratrol to ER+ breast cancer cells was shown to induce apoptosis while inhibiting Akt activation [64].

Lipins are multifunctional proteins involved in lipid metabolism. Lipins can act as enzymes by dephosphorylating phosphatidic acid to diacylglycerol. Lipins can also function as co-transcriptional regulators. The role of lipin-1 in rapamycin sensitivity has recently been investigated. Lipins have been observed to be upregulated in certain tumours including breast cancers. Lipins stimulate the proliferation of breast tumour lines as knock-down of lipin-1 reduced proliferation of breast cancer cells but not of normal cells. Knock-down of lipin-1 resulted in activation of RhoA which suppressed cell migration. Autophagy was also induced when lipin-1 was suppressed. Propranolol is a sympatholytic nonselective beta blocker which will inhibit lipins. Suppression of lipins with propranolol increased sensitivity of the cells to rapamycin [65].

$p27^{Kip1}$ has recently been shown to be a predictive biomarker for the response to rapamycin in certain patient-derived xenograft (PDX) models. Breast cancer cells which expressed high levels of $p27^{Kip1}$ were observed to be sensitive to rapamycin. Rapamycin treatment was determined to decrease phosphorylation of p70S6K and 4E-BP1 [66].

PDX breast cancer models are being developed by many investigators and there are some models commercially available [67, 68]. All the human intrinsic breast cancer subtypes are represented in various breast PDX models. Many small molecule inhibitors (e.g., Bcl-2 inhibitors), drugs such as rapalogs and hormonal-based therapies and others either have been or will be evaluated in PDX models of breast cancer [69, 70].

p70S6K and 4E-BP1 are two key downstream substrates of mTORC1. Knock-down of both p70S6K and 4E-BP1 resulted in a transforming growth factor-β (TGF-β) dependent G_1 arrest in the TNBC MDA-MB-231 cell line. Nanomolar concentrations of rapamycin led to inhibition of p70S6K phosphorylation in MDA-MB-231 cells; however, much higher doses (micromolar) were required to inhibit 4E-BP1 phosphorylation, which resulted in the liberation of eIF4E to promote protein translation. Micromolar concentrations of rapamycin were required to induce G_1 arrest indicating the importance of 4E-BP1. G_1 arrest was determined to be increased by TGF-β signalling and downregulation of Rb phosphorylation by p70S6K and 4E-BP1 respectively [71].

Phorbol esters (PMA) can induce either proliferation or cell cycle arrest depending on the cell type and culture conditions. PMA will hyperactivate the Raf/MEK/ERK pathway in SKBr3 breast cancer cells which in turn induces $p21^{Cip-1}$, cell cycle arrest, and cellular senescence (geroconversion). mTOR and p70S6K were involved in geroconversion. PMA has been shown to induce cell cycle arrest while constitutively active mTOR mediated geroconversion. Rapamycin suppressed geroconversion and maintained quiescence. PMA elicited its effects via phosphorylation of p70S6K on T389 and S6 on S240/244, which was inhibited by rapamycin treatment. In contrast, in the presence of PMA, phosphorylation of p70S6K on T421/S424 and S6 on S235/236 were rapamycin-insensitive. These studies indicated that rapamycin can decrease geroconversion induced by PMA without preventing PMA-induced cell cycle arrest [72].

Rapamycin and autophagy

The effects of rapamycin and the anti-malarial drug hydroxychloroquine on autophagy in cancer patients have been examined. Rapamycin and chemotherapy are known

to enhance autophagy while hydroxychloroquine may inhibit it. Analysis of a limited patient cohort suggested that rapamycin and hydroxychloroquine treatment in combination with chemotherapy resulted in the classification of autophagy as an oncotarget [73].

Other approaches to targtet mTORC1 activity – mTOR inhibitors

AZD2014 is a novel mTOR kinase inhibitor which blocks the activity of both mTORC1 and mTORC2. It has proved to be more effective in inducing growth inhibition in several breast cancer cell lines than everolimus. AZD2014 has been examined in combination with fulvestrant. This combination resulted in tumour growth inhibition or regression in ER+ breast cancer models. The effects of AZD2014 have also been examined in the CTC174 explant model which has a mutated ER. AZD2014 is currently in phase II clinical study development [74].

AZD2014 is being examined in at least six clinical trials with breast cancer patients. Some are in combination with: fulvestrant (NCT01597388 and NCT02216786), the cyclin-dependent kinase4/6 inhibitor, palbociclib (IBRANCE®) and fulvestrant (NCT02599714), the MEK inhibitor Selumetinib (NCT02583542), the oral Akt inhibitor AZD5363 (NCT02208375) or with various other drugs to determine the efficacy of high throughput genome analysis as a therapeutic decision tool (NCT0229999).

A phase I dose-escalation study has been performed with the mTORC1/mTORC2 kinase inhibitor CC-223 in cancer patients with either advanced solid tumours or multiple myeloma. Suppression of downstream targets of mTORC1/mTORC2 was observed in patient biopsies. This phase I study concluded that treatment with CC-223 was tolerable, with manageable toxicities and antitumour activity including regression [75].

Rapalogs and bone cancer pain

The PI3K/PTEN/Akt/mTORC1 pathway has also been shown to be involved in bone cancer pain. Rapamycin was shown to prevent protein kinase C epsilon (PKC-epsilon) and protein kinase A (PKA) normally induced by morphine treatment. Suppression of mTORC1 may be an approach to block bone pain in certain breast cancer patients who exhibit activation of PI3K/PTEN/Akt/mTORC, PKC-epsilon and PKA [76].

Combining PI3K and dual PI3K/MTORC1 inhibitors

The effects of combining the PI3K inhibitor pilaralisib or the PI3K/mTOR dual inhibitor voxtalisib with letrozole has been examined in phase I/II clinical trials in HR+, HER2− breast cancers that were refractory to nonsteroidal AI therapy. Interestingly, in these studies, no association between PI3K pathway mutations and efficacy was observed. These studies indicated that AI and PI3K or PI3K/mTOR inhibitors may be combined to potentially treat endocrine therapy-resistant HR+, HER- metastatic breast cancer patients [77].

The effects of the dual PI3K/mTOR inhibitor NVP-BEZ235 and either an autophagy inhibitor or autophagy gene knock-down have been examined in MCF-7 cells. Combining NVP-BEZ235 and autophagy inhibitors were shown to result in increased growth inhibition [78].

Summary

HR+ breast cancer patients are often treated with endocrine therapies. However, a large proportion of the patients will develop resistance to endocrine therapy and disease progression occurs. Increased PI3K/PTEN/Akt/mTORC1 pathway activity is observed in patients who underwent either endocrine or HER2−targeted therapies. This review has focused on the recent studies on targeting the PI3K/PTEN/Akt/mTORC1 pathway in HR+ breast cancer patients which have become resistant to AIs. Over the past decade, it has become apparent that certain ER+ breast cancer patients may become resistant to nonsteroidal AIs. Part of their resistance may be due to activation of the PI3K/PTEN/Akt/mTORC1 pathway, which is a key pathway involved in proliferation and the prevention of apoptosis. Rapalogs which inhibit mTORC1 were determined to block the AI resistance documenting the importance of this pathway in AI resistance in HR+ breast cancers. Various rapalogs have been examined in clinical trials and one of the most promising is everolimus. This review has also summarized the effects of rapalogs on other breast cancer types including HR-/HER2+ breast cancer. In addition, more novel inhibitors which target the kinase activity of mTOR and suppress both mTORC1 and mTORC2 may be more effective in suppressing AI resistance in combination with AIs. Other drugs and natural products which when combined with rapalogs may eventually be useful in breast cancer therapy (e.g., metformin and resveratrol) have been discussed.

In summary, there are a large number of clinical trials which will determine the effects of everolimus on breast cancer therapy. There are numerous clinical trials listed on ClinicalTrials.gov for the everolimus and breast cancer therapy https://clinicaltrials.gov/ct2/results?term=%22everolimus%22+AND+%22breast+cancer%22&Search=Search. In Table 1, clinical trials with breast cancer patients which examine the effects of rapalogs combined with agents that target HER2 and/or epidermal growth factor receptor (EGFR) or insulin-like growth factor receptor (IGF-1R) and hormonal-based therapy are presented. In Table 2, clinical trials with breast cancer patients which examine the effects of rapalogs combined with chemotherapeutic drugs, PI3K/mTOR kinase inhibitors and metformin are presented. The diverse array of drugs being combined with rapalogs documents the intensity of developing effective approaches to treat breast cancer. These numerous clinical trials and basic research studies point to the significant roles that specific targeted therapy has had on breast cancer treatment which may be extended to other tumours, especially hormonal-responsive cancer.

Competing Interests

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