The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers

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***Abstract*:** The phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) pathway is a complicated intracellular pathway, which leads to cell growth and tumor proliferation and plays a significant role in endocrine resistance in breast cancer. Multiple compounds targeting this pathway are being evaluated in clinical trials. These agents are generally well tolerated and can be used in combination with targeted therapies, endocrine therapy or cytotoxic agents. The identification of subtypes of tumors more likely to respond to these therapeutics cannot be overemphasized, since breast cancer is a very heterogeneous malignancy. Activation of pathways such as KRAS and MEK can act as escape mechanisms that lead to resistance, thus a combination of agents targeting multiple steps of the intracellular machinery is promising. There is evidence that tumors with PIK3CA mutations are more sensitive to inhibitors of the PI3K pathway but this has yet to be validated. Large clinical trials with correlative studies are necessary to identify reliable biomarkers of efficacy.

Keywords: Akt, breast cancer, endocrine resistance, everolimus, mammalian (or mechanistic) target of rapamycin, phosphoinositide 3 kinase, PIK3CA, phosphatase and tensin homolog

Introduction

It is estimated that one in eight women will be diagnosed with breast cancer during their lifetime. Even though advances in cytotoxic chemotherapy and targeted therapies in the past few decades have led to improved survival rates, more than 40,000 patients die from breast cancer annually in the USA [Siegel *et al.* 2013]. The phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) pathway has been associated with resistance to endocrine therapy, human epidermal growth factor receptor 2 (HER2)-directed therapy and cytotoxic therapy in breast cancer [Nahta, 2012; Paplomata and O'Regan, 2013]. Multiple inhibitors of the PI3K/Akt/mTOR pathway are in preclinical development or are already in clinical trials. There are promising data indicating that rapalogs or inhibitors of PI3K/Akt are active in breast cancers. Everolimus is a rapamycin analog and inhibitor of mTOR, which is currently the only compound approved for the treatment of hormone receptor (HR)-positive, HER2-negative metastatic or locally advanced breast cancer.

The PI3K/Akt/mTOR pathway

PI3K/Akt/mTOR is a major intracellular signaling pathway, which responds to the availability of nutrients, hormones and growth factor stimulation and has been well established to play a very significant role in tumor cell growth and proliferation. The central role in this pathway is played by the PI3K heterodimer, which belongs to the class IA of PI3Ks. The heterodimer consists of two subunits, with the regulatory subunit (p85) regulating the activation of the catalytic subunit (p110) in response to the absence or presence of upstream stimulation by growth factor receptor tyrosine kinases (RTKs) [Cantley, 2002]. Each subunit has different isotopes in mammals and their respective genes encode these. Namely, p110α, p110β and p110δ subunits are encoded by PIK3CA, PIK3CB and PIK3CD, while the regulatory subunit is encoded by PIK3R1, PIK3R2, PIK3R3 [Engelman *et al.* 2006].

The PI3Ks phosphorylate phosphatidylinositol 4,5 bisphosphate, or PIP_2, to phosphatidylinositol 3,4,4-triphosphate, or PIP_3, which in turn leads to the phosphorylation of Akt, a serine/threonine

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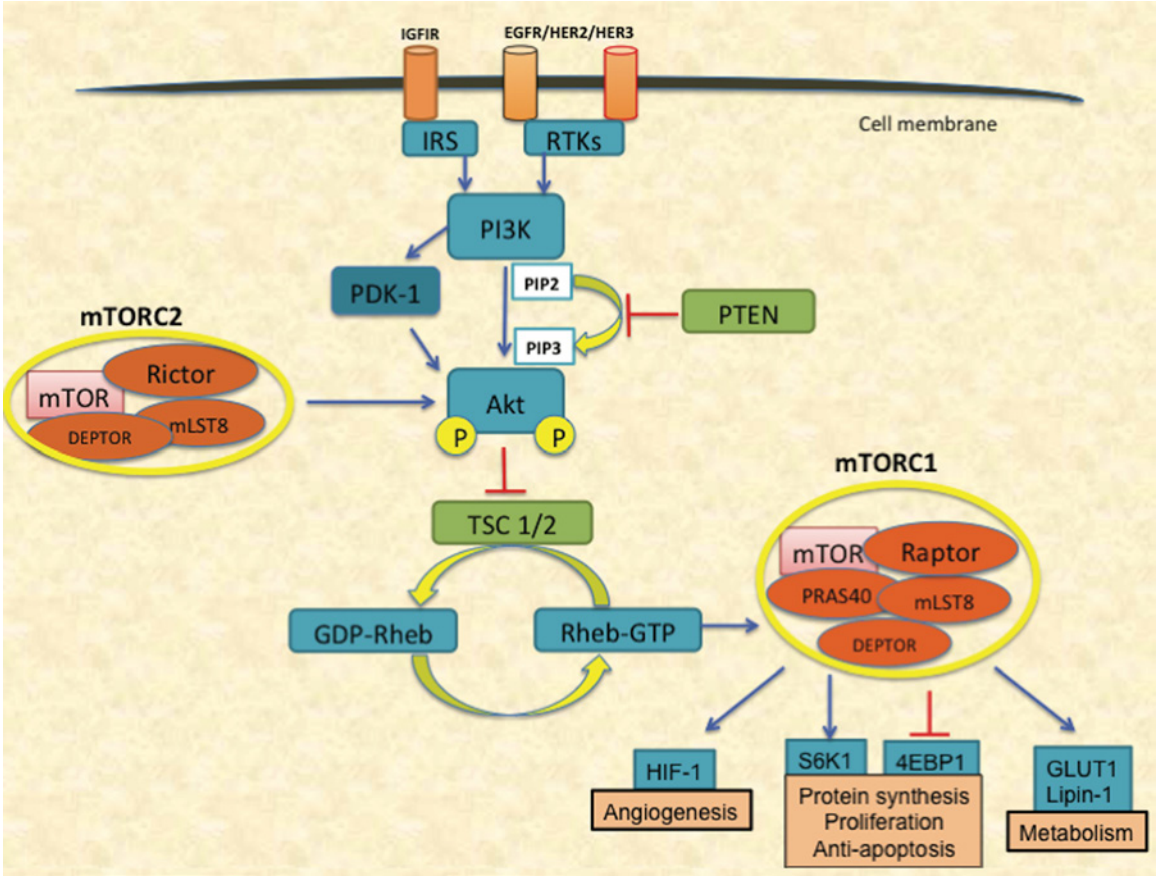

Figure 1. Illustration of the PI3K/Akt/mTOR pathway. The PI3K/Akt/mTOR pathway is a major intracellular network that leads to cell proliferation. The activation of Akt inhibits TSC, which acts as a GTPase activating protein for Rheb. mTORC1 stimulates protein synthesis, metabolism and cell growth via modulation of S6K1 and 4EBP1. mTORC2 activates Akt, which then inhibits the proteolysis of Cyclin D1/E. PTEN and TSC are significant tumor suppressors (shown in green). 4EBP1, eukaryotic initiation factor 4E binding protein 1; Akt, protein kinase B; Glut1, glucose transporter 1; GTPase, guanosine triphosphatase; HIF-1, hypoxia inducible factor 1; IRS, insulin receptor substrate; mTORC1/2, mammalian target of rapamycin complex 1/2; PI3K, phosphatidylinositol 3 kinase; PTEN, phosphatase and tensin homolog; S6K1, S6 kinase 1; TSC, tuberous sclerosis.

kinase, which has impact on cancer cell cycling, survival and growth [Zhao and Vogt, 2008]. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is an important tumor suppressor, which has the opposite action and dephosphorylates PIP_3 into PIP_2 [Maehama and Dixon, 1998]. The loss of PTEN and PIK3CA mutations, which most commonly involve exons 9 and 20, are among the most common aberrations seen in human malignancies, including breast cancer [Samuels and Velculescu, 2004; Cancer Genome Atlas Network, 2012]. It has been recently suggested that Akt-independent activation of the PI3K pathway can occur and that Akt-independent PIK3CA mutations can lead to tumorigenesis [Zhang *et al.* 2012; Bruhn *et al.* 2013].

mTOR is a serine/threonine protein kinase, which is found downstream of PI3K and Akt. mTOR refers to two different complexes, mTORC1 and mTORC2, which have different modes of action. mTORC1 is the target of rapamycin and rapamycin analogs. Even though mTORC1 is much better studied and characterized, now it is also believed that mTORC2 is inhibited by these agents in sufficient doses and that it also affects cell metabolism and cancer cell growth (Figure 1) [Sarbassov *et al.* 2006; Wander *et al.* 2011].

mTORC1 is a complex which consists of Raptor, mLST8 and proline-rich Akt substrate 40 (PRAS40). mTORC1 is activated by Akt via the inhibition of tuberous sclerosis 1/2 (TSC1/2), a tumor suppressor and heterodimer of tuberin and

hamartin, which acts as a guanosine triphosphatase activating protein for Rheb-GTP. Akt phosphorylates TSC2 at the serine 939 and threonine 1462 sites, thus inhibiting TSC1/2; it also phosphorylates PRAS40, thus stimulating mTORC1. mTORC1 affects the cell metabolism and leads to cell anabolic growth via its action on 40S ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein (4EBP1) [Kenerson *et al.* 2002; Dowling *et al.* 2010; Holz, 2012].

Clinical trials with mTOR inhibitors

Rapamycin (sirolimus) was the first available mTOR inhibitor, used as an immunosuppressant in transplant recipients. Novel mTOR inhibitors are now available, which have improved pharmacokinetic and pharmacologic properties. Temsirolimus has been approved as a weekly intravenous infusion for the treatment of renal cell carcinoma. Everolimus is an oral mTOR inhibitor that is approved by the US Food and Drug Administration (FDA) in postmenopausal women with HR-positive breast cancer. Everolimus is also approved in advanced renal cell carcinoma, neuroendocrine tumors of the pancreas and subependymal giant cell astrocytoma. Multiple trials have evaluated mTOR inhibitors in various settings in breast cancer.

HR-positive breast cancer

The PI3K/Akt/mTOR pathway has been implicated in endocrine resistance. Preclinical studies have shown that Akt can activate the estrogen receptor (ER) pathway independent of estrogen availability and that the combination of mTOR inhibitors with endocrine therapy can overcome endocrine therapy resistance [Paplomata *et al.* 2013].

The HORIZON trial, a phase III trial which evaluated the addition of temsirolimus to letrozole in the first-line setting in patients with HR-positive breast cancer, failed to demonstrate a significant improvement in progression-free survival (PFS) for patients treated with the mTOR inhibitor [Wolff *et al.* 2013]. These results contrast with the encouraging results from the BOLERO-2 trial. This randomized, phase III trial evaluated the addition of everolimus to exemestane in postmenopausal women with advanced HR-positive breast cancer who experienced disease relapse or progression on nonsteroidal aromatase inhibitors (AIs) [Baselga *et al.* 2012]. At an interim analysis, the study met its primary endpoint and showed that the addition of everolimus significantly improved PFS in the patients treated with the combination. By local assessment, PFS improved to 6.9 months *versus* 2.8 months in patients receiving placebo (hazard ratio 0.43, $p < 0.001$), while by central assessment, PFS was 10.6 months *versus* 4.1 months respectively (hazard ratio 0.36, $p < 0.001$). More adverse events were reported in the patients receiving everolimus and more patients receiving the combination discontinued the treatment (19% *versus* 4%). The most common adverse events were stomatitis, fatigue, diarrhea, cough and hyperglycemia. A recent updated analysis of the BOLERO-2 study continued to demonstrate the benefit in PFS and also showed fewer deaths in the patients receiving everolimus plus exemestane, though overall survival data are not yet mature [Piccart-Gebhart *et al.* 2012].

The TAMRAD study evaluated the addition of everolimus to tamoxifen in an open-label, phase II study. The primary endpoint was clinical benefit rate (CBR), which was significantly improved in patients receiving everolimus (CBR 61% *versus* 42%, $p = 0.045$). This study also showed that time to progression (TTP) was improved and the risk of death was decreased by 55% in the combination arm. An unplanned analysis demonstrated that patients with acquired resistance to endocrine therapy by the trial criteria obtained more benefit from everolimus compared with patients with cancers deemed to be intrinsically resistant to endocrine agents, though this finding requires confirmation. The toxicities were consistent with prior reports and were most commonly stomatitis, rash, anorexia and diarrhea [Bachelot *et al.* 2012].

Finally, a neoadjuvant study randomized postmenopausal women with early stage, operable HR-positive breast cancer to 4 months of treatment with letrozole plus everolimus or placebo. The clinical response by palpation, which was the primary endpoint, was significantly higher in the everolimus arm. The study also demonstrated that everolimus had a bigger impact on Ki67 downregulation, which has been demonstrated to predict long-term outcome [Dowsett *et al.* 2005, 2007].

These studies demonstrate that mTOR inhibition may play a significant role in HR-positive and endocrine-resistant breast cancer.

A key question is when patients require the addition of everolimus to endocrine therapy. The lack of benefit noted with temsirolimus in the first-line setting perhaps suggests that patients with endocrine-resistant cancer may be more likely to benefit from inhibition of mTOR with endocrine therapy. This hypothesis is supported by the fact that the median PFS for patients in the control arms of BOLERO-2 and TAMRAD was less than 6 months, suggesting that the majority of patients accrued had endocrine-resistant cancers. Additionally, both studies enrolled patients who had previously been treated with an AI and were essentially excluded from the HORIZON study, in which only approximately 40% of patients had received adjuvant endocrine therapy. Suboptimal dosing and schedule of administration may have also played a role in the results of the HORIZON study. It is important to identify which patients are most likely to benefit from everolimus given the added toxicity compared with endocrine therapy alone.

HER2-overexpressing breast cancer

The PI3K/Akt/mTOR pathway has been implicated in trastuzumab resistance in HER2-overexpressing breast cancer. Preclinical studies indicate that inhibitors of the pathway can act synergistically with trastuzumab in resistant cells [Nahta, 2012].

In a phase Ib study of everolimus in combination with paclitaxel and trastuzumab in patients with trastuzumab-resistant breast cancer, the combination had an overall response rate (ORR) of 44% [Andre *et al.* 2010]. Another phase Ib study evaluated everolimus with vinorelbine and trastuzumab and the combination had an ORR of 19%; this study also established the dose of 5 mg of everolimus daily when used in this combination [Jerusalem *et al.* 2011].

A phase II study, which evaluated everolimus with paclitaxel and trastuzumab in 55 patients, found that the ORR was 21.8%, the clinical benefit rate was 36.4% and the median PFS was 5.5 months. The median estimated overall survival was 18.1 months [Hurvitz *et al.* 2013].

The BOLERO-3 trial was a phase III study of everolimus in combination with cytotoxic therapy in patients with HER2-overexpressing locally advanced or metastatic breast cancer, who had previously been treated with a taxane and whose cancers were resistant to trastuzumab. Resistance to trastuzumab was defined as recurrence within 12 months of adjuvant therapy or 5 weeks within treatment of metastatic disease. The patients were randomly assigned to receive everolimus 5 mg/day orally or placebo, in combination with weekly vinorelbine and trastuzumab. The primary endpoint was PFS by local assessment. The trial showed that everolimus improved the PFS from 5.78 to 7 months, with a hazard ratio of 0.78 ($p = 0.0067$). Subset analysis interestingly showed a significant improvement in PFS in patients with HR-negative cancers, but not with HR-positive cancers. There is evidence that documented crosstalk between the ER and the HER2 pathways allows ER to act as an escape pathway when HER2 but not ER are inhibited [Nahta, 2012]. This suggests that in a subset of cancers that are both HR-positive and HER2-positive, inhibition of ER may be critical and improve outcome. Survival data from this study are still immature [O'Regan *et al.* 2013].

It should be noted that the everolimus dose used in the BOLERO-2 study was 10 mg daily, which is the recommended dose in combination with endocrine therapy; however, the BOLERO-3 used a dose of 5 mg daily, which was based on the results of the phase 1b study by Jerusalem and colleagues [Jerusalem *et al.* 2011].

These studies suggest that there may be a role for mTOR inhibition in HER2-overexpressing tumors resistant to trastuzumab. The setting in which everolimus may be used remains unclear, especially with the approval of ado-trastuzumab emtansine (T-DM1), lapatinib and pertuzumab. In contrast to other randomized trials in the trastuzumab-resistant metastatic setting, the BOLERO-3 trial enrolled patients who had previously received lapatinib.

Ongoing studies

Multiple much awaited studies with mTOR inhibitors in patients with breast cancer are currently ongoing. The BOLERO-1 trial [ClinicalTrials.gov identifier: NCT00876395] has completed accrual; this is a phase III, randomized, double-blind, placebo-controlled study, which is evaluating everolimus in combination with trastuzumab and paclitaxel in HER2-overexpressing advanced/metastatic breast cancer as first-line treatment.

The BOLERO-4 study [ClinicalTrials.gov identifier: NCT01698918] is a phase II, open-label,

single-arm trial, which treats patients with ER-positive, HER2-negative advanced/metastatic disease with everolimus and letrozole. Upon progression, patients are offered continuation of everolimus in combination with exemestane. This study is currently recruiting.

BOLERO-6 [ClinicalTrials.gov identifier: NCT01783444] is a phase II, open-label study of everolimus with exemestane *versus* everolimus alone *versus* capecitabine. This study will compare the FDA-approved combination of everolimus and exemestane against everolimus alone or chemotherapy in women with ER-positive, HER2-negative breast cancer, which had progressed or recurred on anastrozole or letrozole. This study is currently accruing participants.

A randomized, phase II study of everolimus or trastuzumab in the hormone-refractory setting is currently recruiting patients [ClinicalTrials.gov identifier: NCT00912340]. This trial randomizes patients with HR-positive cancer and low–moderate expression of HER2 to trastuzumab or everolimus in combination with endocrine therapy. Its purpose is to investigate whether trastuzumab or everolimus alone or in combination are effective in metastatic breast cancers resistant to hormonal therapy.

Ridaforolimus (MK-8669) is a novel agent belonging to the class of mTOR inhibitors, which has significant effects on metabolism and cell growth [Rivera *et al.* 2011]. The results of three studies of ridaforolimus in ER-positive and HER2-positive breast cancer are awaited [ClinicalTrials.gov identifiers: NCT01234857, NCT01605396, NCT00736970].

The role of PI3K/Akt inhibitors

Multiple PI3K or Akt inhibitors are currently in clinical or preclinical studies but no agent is currently FDA approved. Wortmannin is a fungal metabolite and potent pan-specific irreversible PI3K inhibitor, which targets the p110 subunit. Wortmannin was found to inhibit cell growth in several cancer cell lines [Powis *et al.* 1995] and it has also been found to potentiate chemotherapy effects [Ng *et al.* 2000]. LY294002 was the first developed PI3K inhibitor. It has the same mode of action as wortmannin and has been used extensively in preclinical studies in which it has been found to enhance cytotoxic therapy in various tumors [Ng *et al.* 2000; Hu *et al.* 2002; Nguyen *et al.* 2004; McDonald *et al.* 2010]. A synthetic derivative of wortmannin, PX-866 interacts irreversibly with the adenosine triphosphate binding site and is a potent inhibitor of PI3K [Ihle *et al.* 2004]. The most common side effects of PX-866 in phase I studies were gastrointestinal toxicity, especially diarrhea, but the drug was generally well tolerated and increased stable disease in patients with advanced solid malignancies [Hong *et al.* 2012]. PX-866 was also evaluated in combination with docetaxel in patients with solid tumors and the combination was found to be feasible, with similar toxicities and promising results [Bowles *et al.* 2013].

Markman and colleagues reported a phase I trial of BGT226, an oral dual PI3K/mTOR inhibitor, in patients with advanced solid tumors. Most common adverse events included nausea (68%), diarrhea (61%), vomiting (49%) and fatigue (19%) [Markman *et al.* 2012].

BKM120, or Buparlisib (Novartis Corporation, East Hanover, NJ, USA), is an oral selective inhibitor of pan-class I PI3K, which equally inhibits class IA PI3Ks but has no activity against class III PI3Ks or mTOR [Maira *et al.* 2012]. The compound was used in a phase I dose escalation study of 35 patients with advanced solid tumors; it was found to be safe and well tolerated and it demonstrated promising tumor activity. Interestingly, up to 20% of patients experienced mood changes [Bendell *et al.* 2012]. BKM120 was combined with trastuzumab in a phase Ib dose-escalation study; the combination was feasible and safe, with side effects representative of the class of PI3K inhibitors [Saura *et al.* 2014].

BKM120 is undergoing multiple clinical trials in breast cancer and is currently being studied in combination with chemotherapy or endocrine therapy (Table 1). The BELLE-2 [ClinicalTrials.gov identifier: NCT01610284] is a phase III randomized, placebo-controlled trial of BKM120 in combination with fulvestrant in women with HR-positive, HER2-negative breast cancer, who are refractory to an AI. This study is evaluating whether the combination of fulvestrant with a PI3K inhibitor will overcome resistance to endocrine therapy. BELLE-3 [ClinicalTrials.gov identifier: NCT01633060] is a phase III, randomized study, which studies BKM120 in combination with fulvestrant in women with HR-positive, HER2-negative advanced/metastatic breast cancer who have previously been treated with an AI

Table 1. Inhibitors of the PI3K/Akt/mTOR pathway and representative ongoing clinical studies.

Target	Compound	ClinicalTrial.gov identifier	Setting
Pan PI3K inhibitors	XL147	NCT01042925	Phase I/II; XL147 with trastuzumab or with paclitaxel and trastuzumab in MBC
	BKM120	NCT01339442	Phase I; BKM120 with fulvestrant; HR+ MBC
		NCT01633060	Phase III; BKM120 with fulvestrant; HR+, HER2–, AI treated and mTOR inhibitor treated MBC
		NCT01572727	Phase II; BKM120 with paclitaxel in HER– MBC
		NCT01610284	Phase III; BKM120 with fulvestrant; HR+, HER2–, MBC which progressed on or after an AI
		NCT01589861	Phase Ib/II; BKM120 and lapatinib in HER2+, trastuzumab-resistant MBC
		NCT01629615	Phase II; BKM120 in triple-negative MBC
		NCT01132664	Phase Ib/II; BKM120 with trastuzumab in HER2+ breast cancer that has failed to respond to trastuzumab
		NCT01300962	Phase I; BKM120 or BEZ235 and capecitabine in MBC
		NCT01816594	Phase II; BKM120 with trastuzumab and paclitaxel in HER2+ primary breast cancer prior to surgery
	GDC-0941	NCT01437566	Phase II; GDC-0941 or GDC-0980 with fulvestrant *versus* fulvestrant in MBC resistant to AI
		NCT01740336	Phase II; GDC-0941 with paclitaxel *versus* paclitaxel with placebo in locally recurrent breast cancer or MBC
		NCT00960960	Phase Ib; GDC-0941 with paclitaxel, with and without bevacizumab or trastuzumab in locally recurrent breast cancer or MBC
	PX-866	NCT00726583	Phase I; solid tumors, completed
PI3K/mTOR Inhibitors	BEZ235	NCT01300962	Phase I; BKM120 or BEZ235 and capecitabine in MBC
		NCT01495247	Phase Ib/II; BEZ235 with paclitaxel in HER2–, advanced breast cancer or MBC
	XL765	NCT01082068	Phase I/II; XL147 or XL765 with letrozole in HR+, HER2– breast cancer refractory to an AI
Selective p110α inhibitors	MLN1117	NCT01449370	Phase I; metastatic solid tumors
	BYL719	NCT01870505	Phase I; BYL719 with letrozole or exemestane in HR+ advanced breast cancer or MBC
Akt inhibitors	Perifosine	NCT00054145	Phase II; MBC, completed
	MK2206	NCT01245205	Phase I; MK-2206 with lapatinib in solid tumors with dose expansion in HER2+ breast cancer
		NCT01281163	Phase Ib; MK-2206 with lapatinib in HER2+ MBC
		NCT01277757	Phase II; MK2206 in advanced breast cancer with PIK3CA mutation, or AKT mutation, or PTEN loss/mutation
		NCT01776008	Phase II; neoadjuvant MK-2206 with anastrozole with or without goserelin in stage 2 or 3 PIK3CA mutant HR+, HER2– breast cancer
	GDC0068 GSK690693	NCT01562275	Phase Ib; GDC-0973 (MEK inhibitor) and GDC-0068 in solid tumors
mTOR inhibitors	Everolimus	NCT00912340	Phase II; trastuzumab or everolimus in hormone refractory, ER+ MBC
	Temsirolimus	NCT01111825	Phase I/II; temsirolimus with neratinib in HER2+ or triple negative MBC
	Ridaforolimus	NCT00736970	Phase II; in combination with trastuzumab in HER2+ trastuzumab-refractory MBC
		NCT01234857	Phase II; in combination with dalotuzumab *versus* standard of care in HR+ breast cancer
		NCT01605396	Phase II; in combination with exemestane with or without dalotuzumab in HR+ breast cancer
	AZD-8055	NCT00973076	Phase I; solid tumors, completed
	OSI-027	NCT00698243	Phase I; solid tumors or lymphoma, completed
	INK-128 (MLN0128)	NCT01058707	Phase I; advanced malignancies

AI, aromatase inhibitor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin complex; PI3K, phosphatidylinositol 3 kinase; PTEN, phosphatase and tensin homolog.

and an mTOR inhibitor. Additionally, BELLE-4 [ClinicalTrials.gov identifier: NCT01572727] is a phase II trial of BKM120 with weekly paclitaxel in patients with HER2-negative locally advanced/metastatic breast cancer. The BELLE trials are currently recruiting and their results are much awaited as they may show a role for PI3K inhibition in the hormone-refractory setting.

SAR245408 (XL147), a pan-PI3K inhibitor, and SAR245409 (XL765), a PI3K/mTOR inhibitor, are currently undergoing phase I studies in solid tumors. A phase I/II study [ClinicalTrials.gov identifier: NCT01082068] is currently evaluating both agents in combination with letrozole.

GDC-0941 is a potent, selective class I PI3K inhibitor that is currently in clinical studies, including a trial, which combines GDC-0941 with fulvestrant [ClinicalTrials.gov identifier: NCT01437566].

Multiple other agents, which are currently in the pipeline and undergoing clinical studies, are summarized in Table 1.

Side-effect profile

The toxicities of mTOR inhibitors have been well described in multiple clinical trials. Stomatitis, rash, hyperlipidemia, hyperglycemia and myelosuppression are among the most common side effects of mTOR inhibitors. Most of these side effects are grade 1–2 and can be managed with dose reductions and supportive treatment. Stomatitis often leads to dose interruptions or reductions, and several studies investigating the optimal treatment and prevention strategies for stomatitis in patients receiving everolimus may soon be available. Noninfectious pneumonitis is a serious and potentially life-threatening adverse event. Since approximately 50% of the patients are asymptomatic, pneumonitis may be detected incidentally during staging scans. The significance of early detection, prompt intervention and treatment with steroids when indicated cannot be overemphasized. It is also generally essential to optimize treatment of hyperglycemia and hyperlipidemia prior to starting an mTOR inhibitor [Paplomata *et al.* 2013].

The BOLERO-2 study reported stomatitis, rash, fatigue and diarrhea in at least 30% of patients in the everolimus arm [Baselga *et al.* 2012]. The grade 3–4 side effects were stomatitis (8%), anemia (6%), dyspnea (4%), hyperglycemia (4%), fatigue (4%) and pneumonitis (3%). The discontinuation rate in the everolimus group was 19%. In the TAMRAD trial, most of the side effects encountered in the everolimus arm were grade 1 or 2, and 20% of patients in the everolimus arm required dose reductions [Bachelot *et al.* 2012].

PI3K inhibitors have been studied less extensively but most common side effects encountered in phase I studies were gastrointestinal toxicity, such as diarrhea, nausea and vomiting. Rash, hyperglycemia and mood changes can also occur. Mood changes have been seen in about one out of five patients and range from anxiety and depression to hallucinations and affective disorder. This is probably a consequence of PI3K inhibition in the central nervous system, since these drugs appear to cross the blood–brain barrier [Bendell *et al.* 2012; Hong *et al.* 2012; Markman *et al.* 2012; Bowles *et al.* 2013]. Psychiatric side effects up to grade 3 have been observed but they respond well to dose reductions, with or without antidepressants or anxiolytics [Saura *et al.* 2014].

Biomarkers

Breast cancer is a very diverse disease and that makes the discovery of molecular biomarkers very meaningful. Apart from the widely recognized histologic subtypes based on HR and HER2 receptor status by immunohistochemistry, gene expression profiling has lead to the distinction of breast cancer into basal-like, ERBB2 positive, normal breast like, luminal A and luminal B. The basal-like tumors have a high expression of cytokeratins 5/6 and 17, laminin and fatty acid binding protein 7; they comprise approximately 15% of invasive cancers; they most often represent the triple-negative tumors and are more commonly associated with BRCA mutations and high grade. The ERBB2 (HER2) positive tumors usually host TP53 mutations and are associated with inferior clinical outcomes. The luminal tumors are HR positive; the luminal A subtype has a strong expression of ER and is also positive for progesterone receptors. The luminal A tumors are most often low grade and well differentiated. The luminal B subtype tumors have a lower expression of ER and they have a higher proliferation index [Perou *et al.* 2000; Sorlie *et al.* 2001, 2003; Kittaneh *et al.* 2013].

The Cancer Genome Atlas Network went a step further to describe the most common mutations

characterizing breast tumors by subtype [Cancer Genome Atlas Network, 2012].

The luminal/ER-positive tumors were found to have the highest frequency of PIK3CA mutations, especially the luminal A subtype. HER2-positive tumors also had a high rate of PIK3CA mutations. PTEN loss was seen less commonly but significantly. PIK3CA, PTEN and AKT1 mutations appeared to be mutually exclusive. Interestingly, PIK3CA and PTEN mutations can coexist in endometrial cancer and other malignancies [Oda *et al.* 2005]. The presence of PIK3CA mutations in ER-positive tumors was not associated with increased phosphorylation of AKT, S6 and 4EBP1, which are markers of activation of the PI3K pathway [Cancer Genome Atlas Network, 2012]. An analysis of breast cancer tumors and cells lines also failed to find a consistent correlation between PIK3CA mutation status and downstream effectors of the PI3K pathway [Stemke-Hale *et al.* 2008].

Biomarkers of response

Levels of pS6K (S6 kinase) and pAkt may be biomarkers predictive of clinical outcomes and response to inhibitors of the mTOR pathway. High levels of pS6K have been associated with worse prognosis, and the levels of pS6K and pAkt predict a good response to rapamycin or rapamycin analogs in breast cancer cells lines and other tumors [Noh *et al.* 2004, 2008; O'Reilly and McSheehy, 2010; Meric-Bernstam *et al.* 2012]. Breuleux and colleagues investigated a variety of tumor cell lines and reported that high levels of phosphorylated AKT, GSK3β and TSC2 also correlated with increased sensitivity to RAD001 (everolimus) [Breuleux *et al.* 2009].

Another potential biomarker is inositol polyphosphate 4 phosphatase (INPP4B), a tumor suppressor that regulates PI3K/Akt. Its deletion may be seen with PTEN loss and it correlates with poor prognosis. Tumors with INPP4B loss may also be candidates for targeting with PI3k inhibitors [Bertucci and Mitchell, 2013].

There are data supporting that cells with PIK3CA mutations or PTEN loss are more sensitive to PI3K/Akt inhibitors [O'Brien *et al.* 2010; Hong *et al.* 2012, Sangai *et al.* 2012]; Janku and colleagues showed the PIK3CA H1047R mutation as an independent factor that predicts response [Janku *et al.* 2013]. Preclinical studies with the pan-PI3K inhibitor BKM120 have provided evidence that the inhibition of PI3K had significant effect on cells carrying mutations of the PIK3CA gene. In these experiments, while cells with PIK3CA mutations were sensitive to the compound, cells with PTEN or KRAS aberrations were not sensitive [Maira *et al.* 2012]. Triple-negative breast cancer subtypes with activated PI3K pathway due to PIK3CA mutations were found to be sensitive to NVP-BEZ235; however again the loss of PTEN did not predict response to NVP-BEZ235 [Lehmann *et al.* 2011].

Wee and colleagues showed that PTEN-deficient cancer cell lines depend on PIK3CB rather than PIK3CA, which does not affect PI3K or cell growth in these cells. Thus, PTEN-deficient cancers may be better treated with pan-PI3K inhibitors rather than selective p110 inhibitors [Wee *et al.* 2008]. PIK3CA mutant tumors resistant to p110a inhibitors may also be treated with the addition of an mTOR inhibitor to a PI3K inhibitor, since the combination can overcome resistance [Elkabets *et al.* 2013]. While PIK3CA mutated breast cancer cells are more sensitive to mTORC1 inhibitor everolimus and mTORC1/2 inhibitor PP242, PTEN loss may not correlate with response since cells with PTEN loss can be resistant to mTOR inhibition. HER2-overexpressing tumors seem to be dependent or 'addicted' to the PI3K pathway and they are sensitive to mTOR inhibition [Weigelt *et al.* 2011; Liu *et al.* 2013]. Finally, it should also be noted that cancers with PIK3CA mutations and low Akt levels do not appear to be dependent on Akt signaling and thus are not expected to respond to Akt inhibitors [Vasudevan *et al.* 2009].

Biomarkers of resistance

Ribosomal S6 kinases RPS6KA2 (RSK3) and RPS6KA6 (RSK4), NOTCH, c-myc activation and mutations of the mitogen-activated protein kinase pathway can be the basis of resistance to PI3K inhibitors. The combination of PI3K inhibitors with MEK or RSK inhibitors may help overcome this resistance [Janku *et al.* 2011, 2012; Muellner *et al.* 2011; Serra *et al.* 2013]. KRAS mutations also lead to resistance to PI3K/mTOR inhibitors since they seem to bypass the PI3K pathway [Ihle *et al.* 2009; Di Nicolantonio *et al.* 2010; Janku *et al.* 2011]. This is more substantial in basal-like/triple-negative breast cancers, in which the combination of MEK inhibitors and PI3K inhibitors appears to be more significant

than other breast cancer subtypes [Hoeflich *et al.* 2009].

Data from clinical studies

A retrospective review of patients with metastatic breast cancer treated with inhibitors of the PI3K/Akt/mTOR pathway reported that patients with PIK3CA mutations treated with a single agent did not have an increased TTP; however, when PI3K/Akt/mTOR inhibitors were combined with endocrine therapy, HER2-directed therapy or chemotherapy, the presence of PIK3CA mutations correlated with increased TTP compared with patients with wild type tumors [Oliveira *et al.* 2012]. In the same analysis, the PTEN status did not seem to have any correlation with clinical outcome, which seems to confirm the results reported by Weigelt and colleagues.

The neoadjuvant trial of everolimus conducted by Baselga and colleagues evaluated core biopsies before treatment and on day 15 in patients treated with letrozole and everolimus or placebo [Baselga *et al.* 2009]. Patients treated with everolimus had a statistically more significant decrease in Ki67 and pS6. Samples were also analyzed in relation to the presence of PIK3CA mutations; patients with mutations in the exon 9 domain of PIK3CA had an improved response to the combination of everolimus with letrozole.

Molecular analyses have been performed from available tissue from the TAMRAD and BOLERO-2 trials, which evaluated the addition of everolimus to endocrine therapy. In the TAMRAD trial, the presence of PI3K mutations, PTEN and pAKT did not influence the response to everolimus. However, the investigators found that everolimus was more effective in patients who had low PI3K expression; additionally, patients with cancers with low LKB1, a known suppressor of mTOR, and high phospho-4E binding protein, which is downstream of mTOR (Figure 1), achieved greater benefit, though the number of specimens evaluated was small. These data suggest that patients who derive the most benefit from everolimus are those in whom mTOR is activated independently of PI3K [Treilleux *et al.* 2013]. Hortobagyi failed to identify specific genomic abnormalities associated with benefit from everolimus [Hortobagyi *et al.* 2013]. Ellard and colleagues, in a study of various schedules of everolimus in patients with breast cancer, found no correlation between PTEN, HER2 and Akt status and clinical outcomes. This could also be due to the low number of patients evaluated [Ellard *et al.* 2009].

In summary, mutations of PIK3CA and PTEN loss are frequent in breast cancer. Multiple preclinical studies have supported that PIK3CA mutations are predictive of sensitivity to inhibitors of the PI3K/Akt/mTOR pathway, while data on PTEN loss have not been consistent. Clinical studies have not confirmed this correlation between mutation status and clinical response. This may be due to the small number of patients included or the heterogeneity of the tumors. It is also possible that discordance of mutational status between primary tumors and metastatic sites affect these outcomes. The conduct of large correlative studies with banking of sufficient tumor samples for mutation analysis is essential in order to identify biomarkers that will guide the management of a malignancy as heterogeneous and complex as breast cancer [Juric and Baselga, 2012].

Conclusion

The PI3K pathway is a very complicated intracellular network that plays a significant role in breast cancer cell growth and proliferation and is implicated in endocrine resistance in ER-positive tumors. Everolimus is the only FDA-approved inhibitor of mTOR in breast cancer but multiple agents are being evaluated in clinical trials. Inhibitors of the PI3K pathway are generally well tolerated and can be used in combination with cytotoxic chemotherapy or other targeted agents. Also very promising is the ability of some of these agents to cross the blood–brain barrier; thus, they may be used to treat brain metastases [Maira *et al.* 2012; Peddi and Hurvitz, 2014]. The identification of tumors most likely to respond to these agents is very important since breast tumors are heterogeneous and activation of pathways such as KRAS and MEK can act as escape conduits that lead to resistance. There is evidence that tumors with PIK3CA mutations are good targets for inhibitors of the PI3K pathway but this has yet to be validated in the clinical setting. Further studies are necessary to identify suitable and reliable biomarkers that will change clinical practice.

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Conflict of interest statement

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