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Breast Cancer

Targeting the Phosphatidylinositol 3-Kinase Signaling Pathway in Breast Cancer

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Describe how PTEN loss, PIK3CA mutations, and AKT dysregulation affect the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling network in human breast cancer.
2. Review the current state of AKT and mTOR inhibitor development, and describe its potential for clinical applications.

ABSTRACT

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) network plays a key regulatory function in cell survival, proliferation, migration, metabolism, angiogenesis, and apoptosis. Genetic aberrations found at different levels, either with activation of oncogenes or inactivation of tumor suppressors, make this pathway one of the most commonly disrupted in human breast cancer. The PI3K-dependent phosphorylation and activation of the serine/threonine kinase AKT is a key activator of cell survival mechanisms. The activation of the oncogene *PIK3CA* and the loss of regulators of AKT including the tumor suppressor gene *PTEN* are mutations commonly found in breast tumors. AKT relieves the negative regulation of mTOR to activate protein synthesis and cell proliferation through S6K and 4EBP1. The common activation of the PI3K pathway in breast cancer has led to

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the development of compounds targeting the effector mechanisms of the pathway including selective and pan-PI3K/pan-AKT inhibitors, rapamycin analogs for mTOR inhibition, and TOR-catalytic subunit inhibitors. The influences of other oncogenic pathways such as Ras-Raf-Mek on the PI3K pathway and the known feedback mechanisms of activation have prompted the use of compounds with broader effect at multiple levels and rational combination strategies to obtain a more potent antitumor activity and possibly a meaningful clinical effect. Here, we review the biology of the network, its role in the development and progression of breast cancer, and the evaluation of targeted therapies in clinical trials. ***The Oncologist*** 2011; 16:404–414

Introduction

The transformation of normal mammary epithelial cells into cancer cells involves a multistep process with alterations in signal transduction pathways that confer important survival and growth advantages to malignant cells [1]. As part of the growth factor receptor (GFR) signaling, the phosphatidylinositol 3-kinase (PI3K) pathway is a key mediator of cell metabolism and cell growth that is affected by genetic aberrancies at different levels, becoming a crucial pathway for cancer development and representing a therapeutic target against breast cancer [2–5]. Understanding the principal effector mechanisms of the PI3Ks and the cross talk with other oncogenic signaling pathways has been the focus of extensive research to develop drugs with clinical efficacy [6].

PI3K Signaling Pathway

Phosphatidylinositol is a component of eukaryotic cell membranes. The inositol head of the phospholipid can be phosphorylated at multiple sites by phosphoinositide kinases (PIKs), which act as signal transducers involved in the regulation of multiple cell functions [7]. The PI3K superfamily has been studied profoundly since the discovery of PI3K activity associated with viral oncoproteins and its role in growth regulation and prevention of apoptosis and other cellular responses [7]. PI3Ks are grouped into classes I, II or III, depending on their subunit structure, regulation, and substrate selectivity. Each class contains various isoforms, class IA being the most studied in cancer [5]. Class IA PI3Ks (PIK3Cα, PIK3Cβ, and PIK3Cδ) are heterodimeric proteins with a regulatory subunit (p85) and a catalytic subunit (p110), that phosphorylate 4,5-phosphoinositide (4,5-PIP_2) and generate the second messenger 3,4,5-phosphoinosite trisphosphate (PIP_3) [7, 8]. The p110s are encoded by the *PI3KCA* gene and are regulated upstream by growth factor binding to tyrosine kinases receptors and G protein-coupled receptors. Activating mutations in the *PI3KCA* gene and the regulator p85 have been identified in breast cancer [9]. Activated RAS protein can interact with p110 and also activate class IA PI3Ks.

The generation of the second messenger 3,4,5-PIP_3 by class IA PI3Ks plays a key role in downstream signaling by several effector proteins including the serine/threonine kinase AKT and PDK1 (phosphoinositide-dependent kinase 1) [10]. The membrane colocalization of both PDK1 and AKT through their pleckstrin homology domains results in phosphorylation at Thr308 and partial activation of AKT kinase. The phosphorylation of Ser473 by PDK2 generates complete activation of AKT [11]. AKT and its isoforms AKT-1, AKT-2, and AKT-3 have cell-transforming properties through the phosphorylation of multiple protein targets including mTOR (mammalian target of rapamycin), Bad, Caspase 9, Tuberin, GSK3b, and forkhead transcription factors involved in cell survival and apoptosis. Signaling through the PI3K/AKT pathway is negatively regulated by the tumor-suppressor gene *PTEN* (phosphatase and tensin homolog) localized in chromosome 10 [12–14].

AKT Downstream Signaling

AKT is a key regulator of a variety of proteins involved in cell proliferation, metabolism, survival, invasion, migration, apoptosis, and DNA repair. To execute this variety of actions, AKT relieves the negative regulation of mTOR mediated by the tumor-suppressor proteins: TSC1 and TSC2 (tuberous sclerosis complex proteins) [15–17]. Activation of mTOR plays a key role in the activation of protein synthesis contributing to the pathogenesis of multiple tumor types. Phosphorylation of TSC2 by AKT inactivates the GTP hydrolysis of the small GTP-binding protein Rheb (ras homologue enriched in the brain), permitting Rheb to remain in the GTP-bound state. Rheb-GTP binds and activates the mTOR kinase domain [18]. The proline-rich AKT substrate (PRAS40) is also a negative regulator of mTOR and it is inactivated by AKT phosphorylation [19, 20]. These findings expose the fundamental role of AKT in the mTOR activation by growth factors in that AKT inactivates two negative regulators of mTOR [21]. The TSC1/2 complex is also regulated by the LKB1-AMPK (AMP-dependent kinase) and MAPK pathways. These pathways are activated based on the nutritional (amino acids) and energy

status of the cell. The convergence of these signals through the TSC1/2 complex allows mTOR to control cell growth and proliferation based on the availability of nutrients and energy sources [22].

mTOR exists in two multiprotein complexes: mTOR complexes 1 and 2 (mTORC1 and mTORC2). mTORC1 complex is composed of mTOR, raptor, mammalian LST8 (mLST-8/GβL), and PRAS40. mTORC1 activation controls protein synthesis by phosphorylating two translational regulatory proteins: eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) and p70 ribosomal protein S6 kinase (S6K1). Raptor binds to S6K and 4EBP1 substrates and presents them to mTOR for phosphorylation [23]. The activation of S6K and 4EBP1 promotes translation initiation for protein synthesis [24–28]. Important proteins for cell cycle control like D-type cyclins, c-myc, and ornithine decarboxylase are also regulated by this complex [28]. mTOR also decreases ribosome biogenesis regulating transcription of ribosomal RNA and the eukaryotic elongation factor 2 kinase. In regulating the initiation and elongation steps, mTOR controls the overall rate of protein synthesis. The capacity of mTOR to regulate protein synthesis explains in part how the tumor-promoting functions of deregulated mTOR may be distributed among multiple targets [29, 30].

mTORC2 complex consists of mTOR, mSIN-1, mLST-8, PRR5 (proctor), and a different scaffolding protein called rictor (rapamycin-insensitive companion of mTOR) [31]. The activation of this complex remains poorly understood; it appears to be through growth factors in an AKT-independent manner [32]. mTORC2 phosphorylates AKT at Ser473 [33], leading AKT activation toward the Forkhead transcription factor FOXO and the apoptosis regulator BAD. mTORC2 also regulates the cell cytoskeleton and cell polarity through the phosphorylation of protein kinase C (PKCα) [31]. Recent studies in cell lines of colorectal cancer have shown that mTOR-associated proteins, Raptor and Rictor, are overexpressed in colorectal cancer cells [34]. The rapamycin-like drugs directly inhibit mTORC1 but not mTORC2. The Rictor protein makes the FRB domain of mTOR inaccessible to the rapamycin–FKBP-12 complex [35] (Figure 1). In some tumor cells, the inhibition of mTORC1 can enhance PI3K/AKT activation. Under normal conditions, the mTORC1 substrate S6K1 delivers a negative feedback signal by phosphorylating insulin receptor substrate 1 (IRS-1), preventing IRS-1 from recruiting PI3K to the receptor for activation [36, 37]. The inhibition of mTORC1 blocks the S6K-mediated negative feedback, resulting in enhanced PI3K/AKT activation that could activate survival pathways as possible means of resistance [38]. Therapeutic inhibition of mTORC2 may therefore potentiate the effect of mTORC1 inhibitors by preventing AKT activation.

PI3K Pathway Aberrations in Breast Cancer

The PI3K pathway has shown to be activated in a diversity of malignancies including breast, colorectal, ovarian, pancreas, brain, endometrium, and other tumor types. After p53, this pathway is considered to be more affected by genetic alterations than any other pathway in cancer [39]. The role of PI3Ks proteins in oncogenesis has been validated by multiple studies [40] showing that aberrations in this pathway are potential causes of cell transformation and, more significant, that PI3K pathway inhibition causes tumor regression [41, 42].

The PI3K signaling network is known to be affected at different levels in human breast cancer [43]. More than 70% of breast tumors have molecular alterations in at least one component of the pathway [44]. Loss of PTEN, *PIK3CA* mutations, and mutations or other aberrations at the level of PDK1, AKT1, AKT2, and p70S6kinase are some of the known mechanisms that activate the pathway [12]. The identification of genomic alterations and their frequency in the different subtypes of breast cancer may predict responsiveness to targeted therapies. Mouse models and in vitro experiments have shown that tumors with PTEN loss or *PIK3CA* mutations are predicted to be more sensitive to PI3K pathway inhibitors [45, 46]. Both *PIK3CA* mutations and loss of the regulatory actions of PTEN enhance AKT-dependent [47, 48] and AKT-independent [45] downstream pathways and are frequently found in breast cancer.

PTEN Loss

PTEN is a tumor-suppressor gene that inhibits the PI3K/AKT/mTOR pathway by cleaving a phosphate group from the PI3K-activated second messenger PIP-3 [49–51]. The lack of its negative regulatory action causes the activation of the PI3K pathway through the phosphorylation of AKT [52]. PTEN loss has been found in many cancers including breast, endometrial, prostate, and thyroid, among others. Initial studies demonstrated a decreased expression or loss of PTEN in up to 33% of breast tumors and a direct relation of this aberrancy with progression of breast cancer [53, 54]. The loss of PTEN occurs through different ways including somatic mutations, loss of heterozygosity, epigenetic modifications, and protein instability and leads to activation of Akt/mTOR-dependent cell proliferation. Cell lines with PTEN deficiency are mainly inhibited by agents targeting mTOR [55–57]. The association of PTEN loss with clinicopathologic markers and prognosis remains unclear; how-

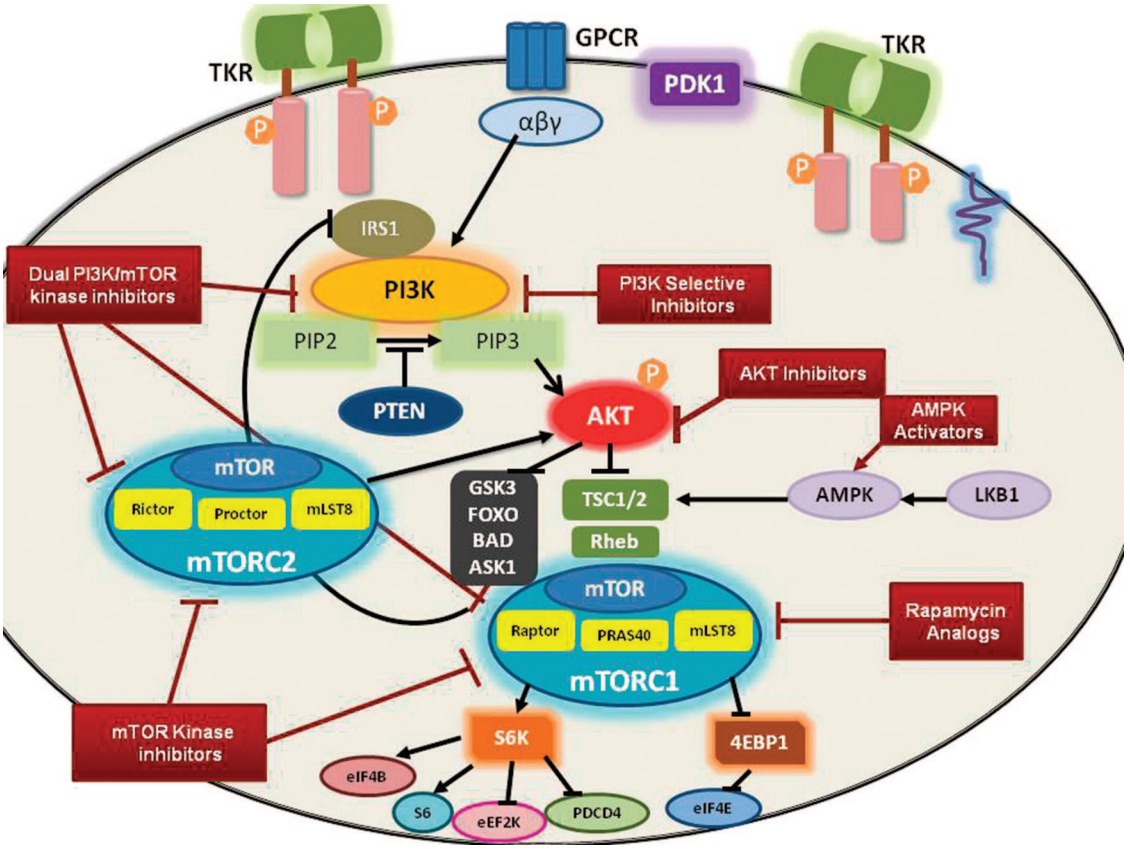

Figure 1. The PI3K/AKT/mTOR signaling network regulates cell survival, proliferation, migration, metabolism, and apoptosis, integrating the growth factor signaling pathway, nutrient status, and other oncogenic pathways. Aberrations at different levels of the network are implicated in breast cancer development and progression. Different therapies targeting the pathway are being developed and included in clinical trials. Arrows represent activation; bars represent inhibition. Abbreviations: 4EBP1, 4E-binding protein 1; AMPK, adenosine monophosphate-activated protein kinase; Bad, BCL2-associated agonist of cell death; FOXO, forkhead box O1; GPCR, G protein-coupled receptor; GSK3, glycogen synthase kinase 3; IRS1, insulin receptor substrate 1; mLST8, mTOR associated protein, LST8 homolog; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol (4,5) biphosphate; PIP3, phosphatidylinositol (3,4,5) triphosphate; PRAS40, proline-rich Akt substrate 40; PTEN, phosphatase and tensin homolog; Rheb, Ras homolog enriched in brain; S6K, ribosomal protein S6 kinase; TKR, tyrosine kinase receptors; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2.

ever, some studies have shown an association of PTEN loss with high tumor grade, tumor size, and negative hormone receptor status [44, 58, 59].

PIK3CA Activation

PIK3CA oncogene mutations are particularly common in breast cancer. The *PIK3CA* gene encodes the p110α catalytic subunit, which plays a key role in the activation of AKT downstream signaling and mammary tumor progression. Activating mutations clustered in the "hot spots" of exons 9 and 20, which correspond to the helical and catalytic domains of p110α, have been reported in up to 26% of breast tumor samples and in 30% of cell lines [60, 61]. In these studies, mutations in exon 20 (catalytic domain) are the most common in breast cancer, in contrast to colorectal cancer where exon 9 mutations are predominant [62]. Several analyses have revealed a direct relation of PI3K activation with lymph node involvement, estrogen receptor (ER), progesterone receptor (PR) positivity, and HER2 overexpression [61, 63]. However, the association with pathologic markers and clinical outcomes is still controversial [44, 64, 65]. Interestingly, there is an inverse relationship between *PIK3CA*-activating mutations and PTEN loss. A recent analysis by Saal et al. reported that, in tumors with *PIK3CA* mutation, only 13% had PTEN loss, whereas 34% expressed PTEN normally [61]. *PIK3CA* and *PTEN* mutations seem to be mutually exclusive and the identification of the driving mutation of each tumor may direct efficacious targeted therapies. Recent data show evidence that *PIK3CA* mutations may contribute to carcinogenesis through both AKT-dependent and AKT-independent mechanisms. In the absence of AKT activation, PDK1 may transmit an alterna-

tive signal that engages downstream substrates such as SGK3 in *PIK3CA* mutant cancer cells [45]. The exact substrates of SGK3 remain to be elucidated [66]. When the AKT-dependent signal is compromised, such as in normal PTEN levels, *PIK3CA* mutations may transduce an AKT-independent signal that engages PDK1 and SGK3 [45].

AKT Dysregulation

The AKT pathway has been found to be dysregulated in a variety of ways in human breast cancer. AKT plays a central role in the pathway and represents an attractive therapeutic target since multiple upstream signaling components converge in AKT. Different studies have demonstrated different roles for the subclasses of AKT in the biological behavior of breast cancer cells. AKT2 activation promotes transition from epithelial to mesenchymal cells, induces secretion of matrix metalloproteinases, and upregulates 1-integrins contributing to tumor invasion and metastasis [66–69]; furthermore, germline deletion of AKT2 in MMTV-ErbB2 mice was shown to decrease lung metastases [70]. Other studies with cell culture systems have shown that the overexpression of AKT1 in breast cancer cell lines results in a decrease in migration and invasion [71, 72]. In transgenic mouse models these two AKT family members have shown to achieve opposing functions in terms of breast tumor metastasis. The hypotheses for the various roles of AKT subclasses include differences in activation levels, interacting partners, downstream substrates, or subcellular localization. The identification of the substrates unique to each isoform of AKT will lead to the therapeutic targeting of specific aspects of tumorigenesis [73–76]. Lopez-Knowles et al. found AKT positivity in 24% of 292 invasive breast cancer patients and a positive association of AKT with high tumor grade, ER and PR negativity, HER2 positivity, and breast cancer–specific death [44].

PI3K Pathway and Breast Cancer Subtypes

Gene expression profiles have classified breast cancer in luminal A, luminal B, HER2-enriched, and basal-like tumors [77] with each subtype reflecting different biology and clinical outcome. A surrogate classification using immunohistochemistry classifies patients based on ER/PR status, HER2, cytokeratin 5/6 (CK5/6), and EGFR status. The frequency and type of PI3K pathway aberrations vary among the different breast cancer subtypes (Table 1) [44, 63]. Each molecular alteration may have a different clinical impact depending on the breast cancer molecular background, the presence of other aberrations, and the treatments received. The genetic heterogeneity of breast cancer and likely different cell origin for each tumor subtype make necessary an independent analysis of the PI3K pathway aberrations by tumor subtype.

Table 1. Aberrancies in the PI3K pathway by breast cancer subtype [44, 49, 63, 94]

Subtype	Aberration	Frequency
All breast tumors	*PIK3CA*	10%–40%
	PTEN	~50%
	AKT	5%–24%
HR-positive tumors	*PIK3CA*	35%–40%
	PTEN	2%–4%
	AKT	2%–3%
Triple negative breast cancer	*PIK3CA*	8%–9%
	PTEN	15%–30%
	AKT	0%
HER2 amplified	*PIK3CA*	20%–25%
	PTEN	30%–40%
	AKT	0%

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; PTEN, phosphatase and tensin homolog.

Hormone Receptor–Positive Tumors

PIK3CA mutations have been found in up to 40% of hormone receptor–positive breast cancer and it is the most frequent aberration of the PI3K pathway found in these tumors [44]. In this subtype, *PIK3CA* mutations have been associated with low mTORC1 signaling and better clinical outcomes in patients treated with tamoxifen monotherapy [78]. The underlying mechanism and downstream signaling pathways to support this favorable association are under investigation. Negative regulatory genes, mTORC1 downregulating feedback mechanisms, and alternative stronger activators are some of the hypotheses postulated [78]. Gene expression profiling analyses are studying the downstream target genes and signaling pathways activated by *PIK3CA* mutations in ERα-positive tumors. Cizkova et al. [79] reported an overexpression of genes involved in the human Wnt signaling pathway, which plays a major role in tumor invasion, metastasis, angiogenesis, and cancer stem cell self-renewal. Some of the genes identified have been linked to tumors with less aggressive features and favorable outcomes. AKT1 mutations seem to be restricted to hormone receptor–positive tumors. AKT1-activating mutations have been linked to initial tumorigenesis with posterior inhibition of invasion and metastasis. In fact, AKT1 may prevent tumor progression and may be associated with good outcomes in this subtype of breast cancer [63].

The critical importance of ER and PR in the develop-

ment and progression of breast cancer [80], and the association of reduced expression of these receptors with poor response to antiestrogen therapy and worse prognosis [81], are well known. The PI3K pathway influences the levels and activity of ER/PR for which this cross talk is a major determinant of both breast cancer progression and response to therapy [82]. The endogenous membrane ER can activate GFRs and PI3K/AKT [83]. The bidirectional cross talk promotes phosphorylation and genomic activation of ER on gene transcription [84–86]. In the presence of hyperactive GFR signaling, as often occurs in breast cancer (e.g., HER2 overexpression), an excessive phosphorylation of ER may diminish the inhibitory effects of the endocrine therapies and lead to endocrine resistance. Cumulative clinical data have shown that patients with HER2- and EGFR-overexpressing tumors have a poorer outcome and are less responsive to tamoxifen [87, 88]. In fact, recent studies using molecular signatures have reported that in ER-positive breast cancer, the GFR/PI3K pathway is associated with lower ER levels and, more importantly, that these levels could be increased by inhibiting the PI3K pathway. The authors suggested that some tumors may rely more on the PI3K signaling than on estrogen for growth and that by blocking the GRF/PI3K pathway these tumors would resort to the estrogen-signaling pathway for survival and restore hormonal sensitivity. Combining the ER blockage and PI3K inhibition might be a more potent treatment strategy [89–91].

Triple Negative Breast Cancer

The basal-like tumors (triple negative for ER, PR, and HER2 and positive for CK5/6 or EGFR) have also shown enhanced PI3K activity mainly through PTEN loss. In gene expression analyses of the main regulators of the pathway, PTEN loss was associated with the basal-like phenotype whereas high PTEN levels were more frequent in luminal A cancers [44]. The loss of PTEN has been reported in approximately 30% of basal-like breast cancers [46] and may play a major role in the pathogenesis of these tumors and poor clinical outcomes of the patients. The aggressive nature and lack of directed therapies against these cancers have promoted a promising growth in the investigation and discovery of potential targets with clinical efficacy. From pharmacogenomic analysis of breast cancer cell lines, genes that constitute the RAS/RAF/MEK signature are the identifiers of the basal-like tumors sensitive to MEK inhibitors. In these studies, loss of the PTEN markedly attenuated the response to MEK inhibition in basal-like tumors. The compensatory upregulation of PI3K/AKT as a survival pathway as a result of PTEN loss is likely the most important mechanism of resistance. The combined treatment with PI3K and MEK inhibitors generated a synergistic effect inhibiting basal-like cell lines. The design of clinical trials with combination therapies including MEK and PI3K inhibitors for this patient population might be a more efficacious approach than single-pathway inhibition therapy [4, 92].

HER2-Amplified Tumors

The *HER2/neu* gene is amplified in 20%–25% of human breast cancer and is associated with aggressive phenotypes and poor outcomes. Despite the major advances in the treatment of HER2-amplified breast cancer with trastuzumab, the development of therapeutic resistance is a current challenge. Only about 30% of HER2-amplified breast cancers respond to trastuzumab therapy. The HER2-positive tumors have shown enhanced PI3K activity mainly through PTEN loss [93]. A combined signature of PTEN loss and *PIK3CA* mutation in HER2-positive breast cancer is a strong predictor of trastuzumab resistance [63]. Recent studies in breast cancer cultured cells have shown that the loss of PTEN or activating mutations in PI3K determine resistance of these cells to trastuzumab, but not to lapatinib [94]. In addition, the identification of tumors with the *PIK3CA* mutation and ER+/HER+ as a group with likely normal PTEN is important since the therapeutic response to trastuzumab is dependent on an intact PTEN [61, 93].

The mechanisms of resistance remain under investigation. In a recent analysis published by Junttila et al., trastuzumab significantly reduced the level of phosphorylation of HER3 and AKT, causing a potent inhibition of the HER3/PI3K/AKT pathway. In these studies, the inhibition of proliferation strongly correlated with the degree of pAKT inhibition and suggested that activators of the PI3K pathway are an important cause of trastuzumab resistance. In cell lines treated with GDC-0941 (agent inhibiting p110α, p110β, and p110δ subunits of PI3K), there was a 40%–85% inhibition of pAKT in all cell lines including trastuzumab-sensitive and -insensitive cells, suggesting a direct correlation between the PI3K/AKT pathway and HER2-positive cells. When these cells were treated with both trastuzumab and GDC-0941, there was a synergistic effect in the inhibition of AKT and downstream targets. The addition of GDC-0941 resulted in inhibition of proliferation in breast cancer cells resistant to trastuzumab because of PTEN loss and activating *PIK3CA* mutations. The combination of the agents was more efficient in the inhibition of the tumors than either of the single agents [95].

In a study correlating the status of multiple components of the PI3K pathway with trastuzumab resistance, Esteva

et al. found that in 137 patients with HER2-positive breast cancer treated with trastuzumab, those who had PTEN-deficient tumors were more likely to be resistant to trastuzumab-based therapy and had decreased overall survival. The combination of other components of the pathway with PTEN loss showed that patients with PTEN−/AKT+ and PTEN−/70S6K+ tumors had more trastuzumab resistance and less overall survival than patients with PTEN loss alone [96]. These data highlight the clinical implications of the PI3K pathway in the mechanisms of resistance to trastuzumab and its potential as a biomarker of prognosis.

Targeting the PI3K Pathway

The PI3K pathway and its upstream and downstream effectors comprise many potential targets for drug development in breast cancer. Agents inhibiting the network at different levels used alone or in combination with chemotherapy, radiation, or other targeted therapies are being evaluated in constantly emerging preclinical and clinical trials (Table 2) [97]. The complexity of the PI3K/AKT/mTOR pathway and the influence of activating alternative cascades and feedback loops have prompted the study of combination therapies and the identification of predictive factors.

PI3K Inhibitors

In the early 1990s the first synthetic PI3K inhibitor LY-294002 was developed [97]. The conjugation of LY-294002 with Arg-Gly-Asp peptides is derived in a prodrug SF-1126 that is in phase I clinical trials as a multimodal Pan-PI3K inhibitor. Phase I clinical trials of BKM120 (a PI3K inhibitor) or BEZ235 (a PI3K/mTOR inhibitor) in combination with endocrine therapy are in progress for postmenopausal patients with hormone receptor–positive metastatic breast cancer. The new generation of PI3K inhibitors is focused on enhancing the potency and the specificity of the compound for particular PI3K isoforms [98]. There are multiple isoform-specific PI3K inhibitors under investigation. CAL-101 (Calistoga Pharmaceuticals Inc.) is a PIK3Cδ selective inhibitor in phase II studies.

As reviewed above, the complexity and multiple interactions of the PI3K pathway make difficult a homogeneous response to targeted treatments. For example, the activation of the pathway by activated RAS mutations limits the effects of single PI3K inhibitors. A more efficacious approach to these tumors is using medications with pan-PI3K inhibitory actions. Pan-PI3K inhibitors include GDC-0941 (Genentech Inc.), which is in a phase I clinical trial, in combination with paclitaxel and bevacizumab for metastatic breast cancer and XL-147 (Exelixis/Sanofi-Aventis) in phase I/II clinical trials alone or combined with trastuzumab and paclitaxel. Pan-PI3K inhibitors with dual PI3K/mTOR inhibitory activity such as XL-765, SF-1126, BEZ-235, GDC-0941, and GSK1059615 are currently in clinical trials for the treatment of breast cancer and other solid tumors. They are thought to work better overcoming the reactivation of the pathway by feedback loops.

AKT Inhibitors

Drug targeting the AKT family has focused on the development of subunit selective inhibiting molecules including ATP competitors, PIP_3 analogs, allosteric inhibitors, pseudosubstrate peptides, and other mechanisms. Preclinical studies have shown that dual AKT-1 and AKT-2 inhibition might be more effective than single inhibition [71]. AKT-3 blockage is more important in tumors like melanoma. The side-effect profile is also isoform-specific and is mainly related to hyperglycemia caused by AKT-2 inhibition [92]. Pan-Akt inhibitors with ATP-competitive properties like AT-13148 and A-443654 are under investigation for clinical development [99, 100].

Allosteric AKT inhibitors disrupt access to the PDK1-dependent AKT phosphorylation site. Compared with ATP-competitive inhibitors, this strategy is more specific and provides better isoform selectivity [101]. The compound MK-2206 (Merck & Co., Inc.) is included in a phase II clinical trial for advanced breast cancer. GSK690693 have entered clinical trials for advanced solid tumors. These compounds are allosteric inhibitors with activity against all three AKT isoforms [102].

mTOR Inhibitors

Since the discovery of rapamycin by Sehgal and colleagues in 1975, extensive work has been done with these compounds as potential agents against cancer [103]. Rapamycin inhibits mTOR [104] regulating the phosphorylation of S6K and 4EBP1/EBP2 for which its direct effect on protein synthesis was elucidated [105]. The mTOR pathway has become an attractive target for drug development against cancer since temsirolimus, a generic analog of rapamycin, was approved for renal cell carcinoma [21, 106]. Preclinical studies in breast cancer have suggested that rapamycin may enhance chemotherapy-induced apoptosis acting in synergisms when combined with standard agents such as paclitaxel, carboplatin, and vinorelbine [106]. The combination of temsirolimus with the aromatase inhibitor letrozole showed some biological and clinical activity in a phase II clinical trial in breast cancer; however, the phase III trial was terminated because of lack of efficacy [107]. Rapa-

Table 2. Agents targeting the PI3K/AKT/mTOR pathway included in clinical trials

Network targets	Agent	Company	Clinical trials	NCT
PIK3CD	CAL-101	Calistoga Pharmaceuticals, Inc.	Phase II	NCT00710528
PIK3CA	BYL719	Novartis	Phase I	NCT01219699
PI3K selective inhibitors	PX-866	Oncothyreon Inc	Phase I	NCT00726583
	GDC-0941	Genentech Inc	Phase II	NCT00960960
	XL-147	Exelixis	Phase I/II	NCT01042925
	BKM-120	Novartis	Phase I	NCT01068483
Multitarget inhibitor Pan-PI3K, mTORC1/2, DNA-PK, HIF-1α	SF1126	Semafore Pharmaceuticals	Phase I	NCT00907205
Dual PI3K/mTOR	XL-765	Exelixis	Phase II	NCT01082068
	BEZ-235	Novartis	Phase I	NCT00620594
	GSK1059615	GlaxoSmithKline	Phase I	NCT00695448
AKT inhibitors	Perifosine	Keryx/AOI Pharmaceuticals	Phase II	NCT00847366
	GSK690693	GlaxoSmithKline	Phase I	NCT00493818
	MK-2206	Merck	Phase II	NCT00963547
Rapamycin analogs	Sirolimus	Wyeth/Pfizer	Phase II	NCT00411788
	Temsirolimus	Wyeth/Pfizer	Phase II	NCT01111825
	Everolimus	Novartis	Phase II/III	NCT01061788
	Ridaforolimus	ARIAD/Merck	Phase II	NCT01234857
mTOR kinase inhibitors	AZD-8055	AstraZeneca	Phase I	NCT00973076
	OSI-027	OSI Pharmaceuticals	Phase I	NCT00698243
	INK-128	Intellikine	Phase I	NCT01058707

Abbreviations: DNA-PK, DNA-dependent protein kinase catalytic subunit; HIF-1α, hypoxia-inducible factor 1, α subunit; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PIK3A, phosphoinositide-3-kinase; α polypeptide; PIK3CD, phosphoinositide-3-kinase, catalytic, δ polypeptide.

mycin analogs temsirolimus (CCI-779) and everolimus (RAD-001) are being used for metastatic breast cancer in combinations with drugs such as capecitabine and exemestane. Ridaforolimus (MK-8669) is in early clinical trials for ER-positive breast cancer.

The majority of preclinical and clinical efforts to target mTOR have involved rapamycin analogs that suppress mTORC1 and do not acutely inhibit mTORC2. The feedback activation of PI3K and AKT limits the efficacy of these compounds [108]. New agents that block this feedback loop by the inhibition of the catalytic activity of TOR with both TORC1 and TORC2 inhibition cause broader suppression of the PI3K/AKT/TOR signaling pathway.

The use of mTOR inhibitors in combination with other targeted agents and chemotherapy may be limited by side effects like myelosuppression, mucositis. and bowel perforation. A new challenge of research is the identification and validation of biologic markers to predict response and to select the high-risk patients that will benefit the most from these therapies [106].

Conclusion

A large amount of clinical data exploring new single and combined therapies to inhibit the PI3K pathway is constantly emerging. Because the PI3K pathway has divergent downstream effects, the identification of the key effectors of the pathway and their presence in the different subtypes of breast tumors will allow the development of ideal targeted therapies with meaningful clinical efficacy. The development of medications with multitarget properties and the identification of potent drug combinations are expected to generate results in the management of breast tumors driven by multiple oncogenic pathways and to overcome resistance by feedback mechanisms. In addition, the heterogeneity of breast cancer makes imperative the identification of biological markers that define molecular profiles for a rational use of PI3K inhibitors.

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